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Opiate receptors and the endorphin-mediated cardiovascular effects of clonidine in rats: Evidence for hypertension-induced μ-subtype to δ-subtype changes

(β-endorphin/opiate receptor subtypes/α_2-adrenergic receptors/nucleus tractus solitarii/blood pressure regulation)

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ABSTRACT **Effects of opiate receptor antagonists on centrally mediated cardiovascular responses to clonidine and β-endorphin were studied in urethane-anesthetized spontaneously hypertensive Okamoto–Aoki rats (SHR), normotensive Sprague–Dawley rats, and Sprague–Dawley rats made hypertensive with deoxycorticosterone pivalate/salt. Microinjection of 270 pmol of naloxone into the nucleus tractus solitarii (NTS) significantly inhibited the hypotensive and bradycardic response to 5 nmol of similarly administered clonidine in both SHR and normotensive Sprague–Dawley rats. In SHR, a similar inhibition was observed after the δ-opiate receptor antagonist ICI 174864, but not after the μ-receptor antagonist β-funaltrexamine (both at 270 pmol, intra-NTS), whereas in normotensive Sprague–Dawley rats, β-funaltrexamine, but not ICI 174864, was an effective inhibitor. The same pattern of differential inhibition was seen when clonidine was given i.v. and the opiate antagonists were given intracisternally in SHR and Sprague–Dawley rats. Intra-NTS microinjection of 280 fmol of β-endorphin caused hypotension and bradycardia, and these effects were similarly inhibited by ICI 174864 in SHR and by β-funaltrexamine in Sprague–Dawley rats. In Sprague–Dawley rats made hypertensive by chronic administration of deoxycorticosterone pivalate and salt, the hypotensive and bradycardic effects of intra-NTS clonidine were inhibited by ICI 174864, but not by β-funaltrexamine, a pattern similar to that in SHR, but different from that in normotensive Sprague–Dawley rats. These results support the hypothesis that β-endorphin release and subsequent stimulation of opiate receptors in the NTS are involved in the cardiovascular effects of clonidine in rats. These results further suggest, however, that hypertension regulates the subtype of opiate receptors mediating these effects.**

Clonidine is a centrally acting antihypertensive agent with high affinity for α_2-adrenergic receptors. Stimulation of α_2-receptors in the brainstem by clonidine-like agents lowers arterial blood pressure (BP) and heart rate (HR) by decreasing sympathetic and increasing parasympathetic outflow to the periphery (1). Morphine and some opioid peptides cause similar effects by interacting with opiate receptors in the same brain region (2). Recent evidence indicates that the effects of central α_2-receptor stimulation are due, in part, to the release of a β-endorphin-like opioid and subsequent stimulation of naloxone-sensitive opiate receptors in the brainstem (3–6). Several laboratories have documented the presence of a naloxone-sensitive component in the cardiovascular effects of clonidine in spontaneously hypertensive rats (SHR), but not in the genetically matched, normotensive Wistar Kyoto rat (6–11). This difference has been interpreted to suggest that hypertension activates or unmasks an endorphinergic depressor mechanism (4–7).

A growing number of reports indicates, however, that the clonidine–naloxone interaction is present in normotensive rats other than Wistar Kyoto rats (6, 12–15), as well as in normotensive animals of other species (16–18), suggesting that the underlying endorphinergic mechanism may have a role in cardiovascular regulation under normal physiological conditions. According to recent evidence, the clonidine-induced release of a β-endorphin-like peptide, as well as the action of this opioid on opiate receptors occurs in the brainstem nucleus of the nucleus tractus solitarii (NTS) (6, 19). The present study was designed to determine the opiate receptor subtype involved in the centrally mediated cardiovascular effects of clonidine. Results support the role of β-endorphin in the effects of clonidine and, further, indicate a hypertension-related change from μ- to δ-type opiate receptors subserving these effects. An account of some of the data has been presented at a symposium (19).

MATERIALS AND METHODS

Animals. Normotensive and hypertensive, 3- to 5-mo-old male rats weighing 300–400 g were used. Normotensive Sprague–Dawley rats and spontaneously hypertensive rats (SHR) of the Okamoto–Aoki strain were obtained from Canadian Breeding Farms of Canada (St. Constant, PQ). The animals were fed normal rat chow, drank tap water *ad libitum*, and were housed under a controlled light/dark cycle of 12:12. To induce hypertension in Sprague–Dawley rats, unilaterally nephrectomized animals were injected weekly with deoxycorticosterone pivalate (10 mg/kg) and received 1% saline in drinking water. The animals became hypertensive within 4–5 weeks of treatment, as tested by measurements of systolic BP of the unanesthetized animals by tail plethysmography (20). Wistar Kyoto rats were not used in this study because the effects of clonidine are naloxone resistant in these animals (4–11).

Anesthesia. Experiments were done in rats anesthetized with urethane, 0.8 g/kg i.v. plus 0.3 g/kg i.p. Urethane was chosen because it provides long-lasting and stable anesthesia with minimal interference with neurally mediated cardiovascular regulatory functions (21). When urethane is administered according to the above protocol, it depresses basal BP less than when it is given by the more common i.p. route (21).

Experimental Protocol. For direct measurement of BP, a polyethylene cannula was inserted into the femoral artery and

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Abbreviations: SHR, spontaneously hypertensive rats; NTS, nucleus tractus solitarii; BP, blood pressure; HR, heart rate; β-FNA, β-funaltrexamine.

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connected to a pressure transducer (Statham, Hato Rey, PR, model P23Db) and polygraph (Grass, model 7). Heart rate was monitored through a tachograph preamplifier (Grass model 7P44B). The animals were then placed into a stereotaxic head holder (David Kopf Instruments, Tujunga, CA), with the head flexed at an angle of 45°. The dorsal surface of the medulla oblongata was exposed by limited craniotomy, and a double-barreled glass microcannula was lowered into the right NTS using a previously published procedure and coordinates (6). One barrel was prefilled with clonidine or β-endorphin, and the other was prefilled with an opiate receptor antagonist for microinjections in volumes not exceeding 200 nl. In some experiments, clonidine was injected i.v. through a cannula in the femoral vein, whereas opiate receptor antagonists were administered intracisternally as described (5). Dynorphin was also administered intracisternally.

Drugs. Drugs used were from the following sources: urethane, clonidine hydrochloride, camel β-endorphin and dynorphin 1–13 (Sigma), naloxone hydrochloride (a gift from Endo Laboratories, Garden City, NY), β-funaltrexamine (Research Biochemicals, Wayland, MA), ICI 174864 [*N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (Aib, aminoisobutyric acid)], a gift from R. J. Rance, Imperial Chemical Industries Pharmaceuticals Division, Alderley Park, U.K.].

Statistical Analyses. The data were analyzed by two-way analysis of variance followed by Duncan's multiple range test (22), or by Student's paired or unpaired *t* tests, as appropriate. A P value of <0.05 was considered to indicate statistical significance.

RESULTS

In agreement with earlier findings, intra-NTS administration of 5 nmol of clonidine to spontaneously hypertensive rats (SHR) caused hypotension and bradycardia that lasted 60–90 min (Fig. 1). A second injection of clonidine 2 hr after the first injection elicited the same decrease in BP and HR, indicating a lack of tachyphylaxis under these conditions (data not shown). However, when the second clonidine injection followed by 10 min the intra-NTS administration of 270 pmol of naloxone, the effects of clonidine were significantly attenuated (Fig. 1 *Left*). Peak BP response to clonidine was inhibited by 60 ± 18%, whereas a dose of naloxone lower by a factor of 10 (27 pmol), tested in separate experiments, only caused a 10 ± 8% inhibition, which was not significant (data not shown). Naloxone blocks all three major subtypes of opiate receptors, with relative potencies of $\mu > \delta \geq \kappa$ (23). To test which of these is involved in the actions of clonidine, we used subtype-selective antagonists. β-Funaltrexamine (β-FNA) is a potent, irreversible antagonist of μ receptors with transient κ-agonist activity and negligible effects on δ receptors (24), whereas ICI 174864 is a selective, reversible antagonist of the δ-receptor subtype (25). When tested in SHR, intra-NTS injection of 270 pmol of ICI 174864 10 min before clonidine significantly inhibited the effects of the latter on both BP and HR (Fig. 1 *Right*), whereas the same dose of β-FNA was ineffective (Fig. 1 *Center*). β-FNA was injected 1 hr before clonidine to minimize interference by its transient agonist activity at κ receptors.

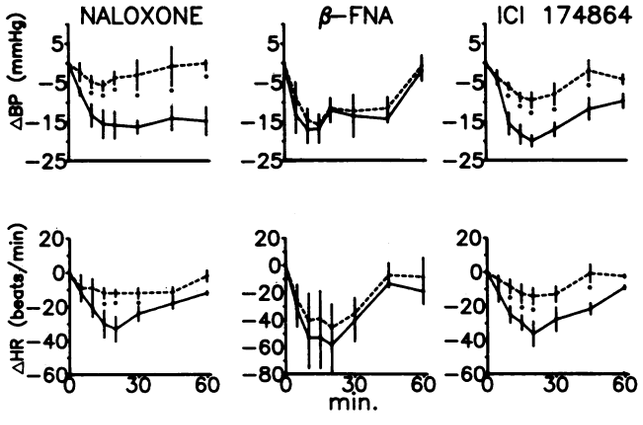

FIG. 1. Inhibition of the cardiovascular effects of clonidine by opiate antagonists in anesthetized SHR. Clonidine (5 nmol intra-NTS) was injected at 0 min before (solid lines) and 10 min after (naloxone or ICI 174864) or 60 min after (β-FNA) the similar injection of 270 pmol of the indicated antagonist (dashed lines). Mean changes in BP (*Upper*) and HR (*Lower*) are shown, with vertical bars representing 2× SE. The number of experiments was four for each antagonist. Baseline BP (mm Hg) without or with the antagonist, respectively, was 110 ± 4 and 105 ± 7 (naloxone), 119 ± 4 and 126 ± 6 (β-FNA), and 110 ± 5 and 111 ± 6 (ICI 174864). Corresponding baseline HR values (beats per min) were 324 ± 10 and 321 ± 5 (naloxone), 332 ± 4 and 322 ± 3 (β-FNA), 348 ± 9 and 351 ± 8 (ICI 174864). ·, Significant difference ($P < 0.05$) from corresponding control clonidine responses.

In preliminary experiments, naloxone was an effective inhibitor of the centrally mediated cardiovascular effects of clonidine in normotensive Sprague–Dawley rats (26). This is confirmed by the data in Fig. 2 *Left*. Naloxone was actually somewhat more potent in Sprague–Dawley rats than in SHR: it reduced the peak BP response to clonidine by 68 ± 6% (270 pmol) or by 34 ± 12% (27 pmol, data not shown). We then tested the effects of subtype-specific antagonists on the clonidine response. Unexpectedly, in the normotensive Sprague–Dawley rat β-FNA was the effective inhibitor (Fig. 2 *Middle*), whereas ICI 174864 did not influence the clonidine response (Fig. 2 *Right*).

To see whether the above drug interactions are relevant for the effects of systemically administered clonidine, in additional experiments clonidine (20 nmol/kg) was injected i.v. before and after the intracisternal administration of 2.7 nmol of the opiate antagonists. The data in Table 1 indicate that the pattern of effects is similar to that seen after intra-NTS drug administration. The effects of clonidine were significantly attenuated by ICI 174864 in SHR, but not in Sprague–Dawley rats. In contrast, β-FNA was ineffective in SHR, but inhibited the effect of clonidine on BP (although not on HR) in

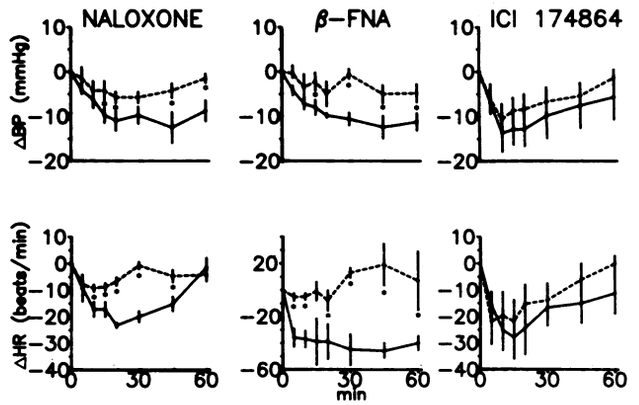

FIG. 2. Inhibition of the cardiovascular effects of clonidine by opiate antagonists in normotensive Sprague–Dawley rats. For explanation and details of the experimental protocol, see the legend for Fig. 1. The number of experiments was four for naloxone, five for β-FNA and seven for ICI 174864. Baseline BP (mm Hg) without and with the antagonist, respectively, was 60 ± 6 and 68 ± 5 (naloxone), 99 ± 6 and 100 ± 3 (β-FNA), and 80 ± 6 and 79 ± 8 (ICI 174864). Corresponding baseline HR values (beats per min) were 300 ± 15 and 312 ± 11 (naloxone), 378 ± 27 and 350 ± 26 (β-FNA), 347 ± 23 and 331 ± 18 (ICI 174864).

Table 1. The effects of intracisternally administered opiate receptor antagonists on cardiovascular responses to i.v. clonidine in SHR and normotensive Sprague–Dawley rats

	β-FNA		ICI 174864	
	Before	After	Before	After
SHR				
Basal BP, mm Hg	136 ± 14	134 ± 7	115 ± 7	130 ± 4
Clonidine*	−15 ± 4	−12 ± 4	−14 ± 2 †	−7 ± 3
Basal HR, beats per min	421 ± 20	379 ± 3	377 ± 16	403 ± 17
Clonidine	−44 ± 4	−32 ± 10	−47 ± 6 †	−16 ± 5
Sprague–Dawley				
Basal BP, mm Hg	95 ± 2	98 ± 4	84 ± 5	91 ± 3
Clonidine	−16 ± 4 †	−5 ± 2	−17 ± 3	−15 ± 2
Basal HR, beats per min	421 ± 14	417 ± 23	362 ± 21	360 ± 20
Clonidine	−50 ± 11	−40 ± 11	−61 ± 11	−45 ± 11

Clonidine, 5 μg/kg, was injected i.v. before and 10 min or 60 min after ICI 174864 or β-FNA, respectively. Means ± SE from four experiments in each group are shown. †, Significant difference between adjacent values.
*Clonidine responses are given in change from basal values listed above.

Sprague–Dawley rats. These findings indicate that while the cardiovascular effects of clonidine have naloxone-sensitive components in both SHR and Sprague–Dawley rats, the opiate receptor subtype involved in these effects is different in the two strains.

Several lines of evidence support the hypothesis that the opioid involved in the naloxone-sensitive effects of clonidine is β-endorphin (4–6, 10, 11, 27). Therefore, we examined the opiate-receptor subtype mediating the cardiovascular effects of β-endorphin itself. In these experiments, 280 fmol (1 ng) of β-endorphin was injected intra-NTS before and after the similar administration of 270 pmol of β-FNA or ICI 174864. The dose of β-endorphin used caused hypotension and bradycardia similar in magnitude and duration to that elicited by clonidine in both SHR and normotensive Sprague–Dawley rats (Fig. 3). Moreover, the pattern of inhibition of these responses was also similar to that seen with clonidine: in SHR, the effects of β-endorphin were inhibited by ICI 174864, but not by β-FNA, whereas the reverse was true in normotensive Sprague–Dawley rats (Fig. 3). These experiments demonstrate that the cardiovascular effects of clonidine and β-endorphin involve activation of the same opiate receptor subtype in the NTS, which appears to be of the μ subtype in Sprague–Dawley rats and the δ subtype in SHR.

The pharmacological profile of the antagonists used and the fact that β-endorphin has high affinity for both μ and δ receptors (23) but is inactive at κ receptors (28) discount the possibility of κ-receptor involvement in the effects of clonidine. Nevertheless, we tested the cardiovascular effects of dynorphin, a putative endogenous ligand for the κ receptor (29). Intracisternal administration of 10 nmol (16 μg) of dynorphin caused a transient pressor response. The peak increase in BP was 24 ± 5 and 14 ± 5 mm Hg, and the peak increase in HR was 61 ± 7 and 41 ± 3 beats per min in SHR and Sprague–Dawley rats, respectively. Lower doses of dynorphin did not cause consistent changes in BP or HR. Thus, the effects of dynorphin are different from those of clonidine.

The difference in opiate receptor subtypes in the NTS of SHR and Sprague–Dawley rats may be genetically determined. Alternatively, it may be related to the difference in BP in the two types of rats. To distinguish between these possibilities, we tested the clonidine–opioid interaction in

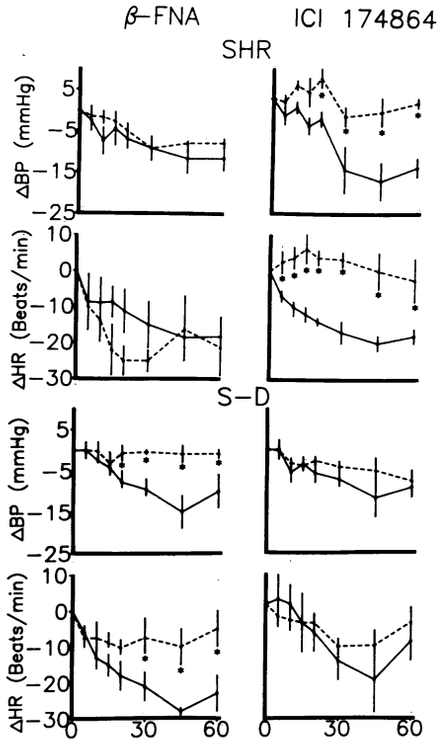

FIG. 3. The effects of β-FNA and ICI 174864 on the cardiovascular response to β-endorphin in SHR and normotensive Sprague–Dawley (S–D) rats. β-endorphin (280 fmol; 1 ng) was microinjected into the NTS of SHR (upper four panels) or S–D rats (lower four panels) before (solid lines) or after the administration of the indicated antagonist (dashed lines). The antagonists (270 pmol) were microinjected into the ipsilateral NTS 10 min (ICI 174864) or 1 hr (β-FNA) before the second β-endorphin injection. *, Significant difference ($P < 0.05$) from the corresponding control β-endorphin response. In SHR, baseline BP (mm Hg) before and after the antagonists were 134 ± 8 and 116 ± 5 (ICI 174864) and 130 ± 8 and 123 ± 3 (β-FNA), and in S–D rats they were 101 ± 7 and 97 ± 4 (ICI 174864), and 102 ± 6 and 95 ± 2 (β-FNA). The corresponding basal HR values (beats per min) in SHR were 377 ± 24 and 348 ± 17 (ICI 174864); 352 ± 10 and 378 ± 12 (β-FNA), and in S–D rats they were 361 ± 20 and 360 ± 15 (ICI 174864) and 406 ± 20 and 351 ± 13 (β-FNA).

Sprague–Dawley rats made hypertensive by the chronic administration of a mineralocorticoid and salt. By the end of the fourth week of treatment, basal systolic BP of the unanesthetized rats rose from 120 ± 8 to 160 ± 10 mm Hg. Earlier experiments have demonstrated opioid involvement in the depressor effects of central α_2-receptor stimulation in such animals (5). Here we tested the interaction between intra-NTS clonidine and similarly administered, subtype-specific, opiate antagonists in urethane-anesthetized, deoxycorticosteroid/salt hypertensive Sprague–Dawley rats. As shown in Fig. 4, clonidine caused hypotension and bradycardia, which were almost twice as large as in normotensive Sprague–Dawley rats (see Fig. 2). Both BP and HR responses to clonidine were significantly attenuated by ICI 174864, but these responses remained unchanged after β-FNA (Fig. 4). Thus, the response pattern in these animals was different from that in normotensive Sprague–Dawley rats, but similar to that seen in SHR.

DISCUSSION

The present findings confirm previous observations that naloxone attenuates the centrally mediated cardiovascular effects of clonidine, not only in SHR (3–11) but also in normotensive rats other than Wistar Kyoto rats (6, 12–15,

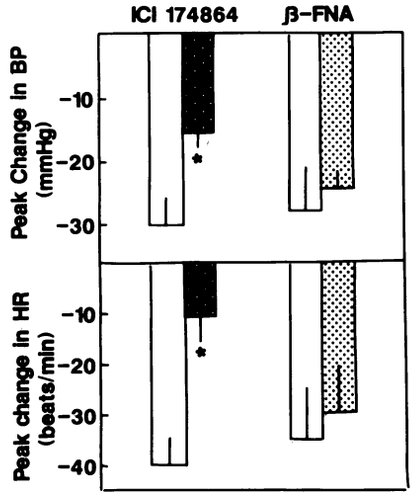

FIG. 4. Inhibition of the cardiovascular effects of clonidine by ICI 174864, but not by β-funaltrexamine in deoxycorticosterone/salt hypertensive Sprague–Dawley rats. Columns and vertical bars represent means ± SEM for peak changes in BP (*Upper*) and HR (*Lower*) in response to 5 nmol of intra-NTS clonidine without antagonist (open bars), after the intra-NTS injection of 270 pmol of ICI 174864 (white dots on black bars), or after the similar administration of 270 pmol of β-FNA (black dots on white). *, Significant difference from control clonidine response. Basal BP values before and after the antagonists were 115 ± 6 and 102 ± 7 mm Hg (ICI 174864) and 119 ± 8 and 124 ± 12 mm Hg (β-FNA). Corresponding basal HR values were 363 ± 6 and 344 ± 16 (ICI 174864) or 348 ± 8 and 345 ± 4 beats per min (β-FNA), respectively.

19). This suggests that opiate receptors are generally involved in mediating the effects of clonidine in rats and the absence of such an interaction in Wistar Kyoto rats (4–11) is a strain-related anomaly. The results also indicate, however, that though in normotensive Sprague–Dawley rats the opiate receptor involved in clonidine effects is of the μ subtype, the same effects are mediated by δ-opiate receptors in animals with either genetic or experimentally induced hypertension. Finally, a similar difference in the subtype of opiate receptors mediating the cardiovascular effects of centrally administered β-endorphin supports the hypothesis that the opioid involved in the effects of clonidine is β-endorphin.

The effects of endogenous opioids are mediated by multiple opiate receptors with different pharmacological properties and tissue distribution (23). The receptor subtypes most commonly distinguished are μ, δ, and κ. β-Endorphin has equally high affinity for μ and δ receptors (23), whereas it is inactive at κ receptors (28). Naloxone is less potent at δ than at μ receptors (23), and the relatively high dose of naloxone required to inhibit the cardiovascular effects of clonidine in SHR (7) has suggested the involvement of δ receptors in these effects. This is supported by the present findings in SHR, where intra-NTS injection of a δ-subtype, but not a μ-subtype, antagonist attenuated the cardiovascular effects of intra-NTS clonidine. Unexpectedly, findings in normotensive Sprague–Dawley rats were the opposite, suggesting the involvement of μ receptors in the same effects. Naloxone was only slightly more potent in normotensive-Sprague–Dawley rats than in SHR, while the *in vitro* affinity of naloxone for μ receptors is ten to twenty times higher than its affinity for δ receptors (23). A possible explanation for this discrepancy may be that the responses inhibited by β-FNA in Sprague–Dawley rats are mediated by a μ_2-type receptor, which has been associated with opiate-induced respiratory depression and muscle rigidity, the naloxone sensitivity of which is lower than the sensitivity of the μ_1-receptor subtype involved in morphine analgesia (30).

Experiments where clonidine was injected i.v. and the opiate antagonists intracisternally revealed a similar involvement of μ receptors in Sprague–Dawley rats and δ receptors in SHR. This not only confirms the strain-dependent difference in opiate-receptor subtypes, but also indicates the importance of the NTS in the cardiovascular effects of systemically administered clonidine in both rat strains. β-Endorphin has been strongly implicated in the cardiovascular effects of clonidine-like agents: clonidine and α-methylnorepinephrine were found to release immunoreactive β-endorphin *in vitro* from brain stem slices of SHR (4), and their cardiovascular effects were inhibited by centrally administered antisera to β-endorphin (5, 10, 15). Intra-NTS injections of low doses of β-endorphin cause hypotension and bradycardia (31), which has been confirmed in the present experiments. The observation that these effects are inhibited by a μ-receptor antagonist in Sprague–Dawley rats and by a δ-receptor antagonist in SHR parallels the findings with clonidine and gives strong support to the notion that the peptide responsible for the naloxone-sensitive component in the effects of clonidine is β-endorphin. The very low dose of β-endorphin used in the present experiments is compatible with the physiological relevance of the observed response. At much higher, pharmacological doses, β-endorphin can elicit pressor responses (1–10 μg intra-NTS, ref. 31), and can increase sympathetic tone (26 μg intracisternally, ref. 32). In the present study, a similar high dose of dynorphin caused a pressor response in both SHR and Sprague–Dawley rats, whereas lower doses did not consistently alter BP and HR. A reported depressor response to intracisternal dynorphin required an even higher dose (33). These findings argue against a proposed involvement of dynorphin in the cardiovascular actions of clonidine (34), at least at the level of the brainstem, and also discount the possible involvement of κ receptors in these effects.

Is the difference in opiate receptors in the NTS related to animal strain, or is it linked in some way to the hypertensive process? We addressed this question by the experiments with deoxycorticosterone/salt hypertensive Sprague–Dawley rats. This model was chosen because, as in SHR, central mechanisms appear to be involved in the hypertension elicited by mineralocorticoids (35). The receptor subtype involved in the effects of clonidine in these rats was δ—i.e., the same as in SHR, but different from that in normotensive animals of the same strain. Sodium can differentially regulate opiate μ and δ receptors (36), and it is possible that the sodium given in conjunction with the mineralocorticoid selectively suppressed μ- and unmasked δ-receptor activity. However, the similar reactivity of SHR and deoxycorticosterone/salt hypertensive rats favors the alternative possibility that the change from μ- to δ-type response is related to the hypertensive state. The NTS is a complex structure containing a multiplicity of cell groups, transmitters, and receptors. Parallel pathways impinging on distinct populations of μ and δ receptors possibly are inversely affected by hypertension, which may account for the apparent change in type of functional receptor. Alternatively, μ and δ receptors have been proposed to be allosterically linked, or even "interconvertible," as suggested by the results of certain morphological (37), biochemical (38, 39), and physiological studies (40, 41) of brain opiate receptors. Earlier studies have documented a similar, inverse regulation of α_1- and β-adrenergic receptor-mediated events in rat heart and liver (42, 43). According to recent evidence, these latter phenomena are due to inverse changes in the coupling of distinct α_1- and β-receptor entities, rather than changes in a single receptor, and the underlying cellular mechanism involves parallel activation of protein kinase C and membrane phospholipase A_2 (44). Although the possibility of a similar regulatory mechanism for μ- and δ-opiate receptors has not

yet been explored, present findings do suggest that inverse regulation of these two receptor subtypes has pathophysiological implications.

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