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No Evidence for Involvement of Endogenous Opioid Peptides in Effects of Clonidine on Blood Pressure, Heart Rate and Plasma Norepinephrine in Anesthetized Rats

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ABSTRACT

Endogenous opioid peptides have been implicated in the cardiovascular depressant actions of clonidine. The effects of clonidine have therefore been examined in anesthetized rats after pretreatment with naloxone, in morphine-dependent rats to determine if cross-tolerance operates and in hypophysectomized rats to determine whether circulating β-endorphin may play a role. In normotensive and spontaneously hypertensive rats, naloxone (2 mg/kg i.v.) did not alter the blood pressure and heart rate response curves to successive doubling doses of clonidine (0.625–10 μg/kg i.v.). In normotensive and spontaneously hypertensive rats made morphine-dependent (3 × 75-mg morphine pellets s.c.), the cardiovascular responses to clonidine were not inhibited but rather enhanced with a greater maximal response of blood pressure and an increase in both the slope and the maximal response of the dose-heart rate response curve. Plasma clonidine levels were similar in normotensive and spontaneously hypertensive rats and corresponding morphine-dependent rats. In hypophysectomized rats, the effect of clonidine on blood pressure was also enhanced with an increase in the maximal response. The reduction in circulating norepinephrine concentrations produced by clonidine was similar in all groups. These results do not support a role for endogenous opioid peptides in the cardiovascular actions of clonidine but do suggest that factors in addition to a reduction in sympathetic nerve activity may be operating.

There is considerable evidence linking the effects of the antihypertensive drug clonidine with opioid-mediated effects. Clonidine is a relatively selective *alpha*-2 adrenoceptor agonist and produces hypotension and bradycardia by activation of inhibitory pathways in the brain, particularly the brainstem (Kobinger, 1978). Clonidine also produces analgesia which can be antagonized by naloxone (Lin *et al.*, 1980) and a withdrawal syndrome, the cardiovascular components of which are alleviated by morphine (Thoolen *et al.*, 1981). Conversely, clonidine reverses symptoms of opioid withdrawal both in experimental animals (Laverty and Roth, 1980; Buccafusco, 1983) and in humans (Gold *et al.*, 1978).

Recently, Farsang *et al.* (1980) reported that naloxone could inhibit and reverse the fall in blood pressure and heart rate produced by clonidine and α-methyldopa in conscious and anesthetized SHR. Both clonidine and α-methylnorepinephrine were reported to increase the release of β-endorphin immunoreactivity from brainstem slices in SHR and it was postulated that *alpha* adrenoceptor agonists may release β-endorphin *in vivo* which in turn would contribute to the fall in blood pressure (Kunos *et al.*, 1981). Further support for this proposition was provided by the demonstration that the hypotension after injection of α-methylnorepinephrine into the nucleus tractus solitarius of anesthetized normotensive rats could be antagonized by naloxone and β-endorphin antibodies (Petty and de Jong, 1982). Naloxone was also reported to antagonize the hypotensive effect of clonidine in humans (Farsang *et al.*, 1982). On the other hand, studies have shown that naloxone fails to alter clonidine-induced changes in blood pressure, heart rate and sympthetic nerve firing in anesthetized cats (Shropshire and Wendt, 1983) and does not change the hypotensive effects of clonidine in normal volunteers (Watkins *et al.*, 1980) or hypertensive patients (Rogers and Cubeddu, 1983).

Because of these conflicting results, we proposed to re-evaluate the possible involvement of endogenous opioid peptides in the cardiovascular effects of clonidine in anesthetized normotensive rats and SHR. In addition to monitoring blood pressure and heart rate responses we have measured concentrations of circulating norepinephrine, a sensitive index of sympathetic nerve activity. Furthermore, we have examined clonidine-induced cardiovascular changes in rats made morphine-dependent and in hypophysectomized rats as well as after pretreatment with naloxone. It has been argued by Sawynock *et al.* (1979)

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ABBREVIATION: SHR, spontaneously hypertensive rats.

that in addition to antagonism by naloxone, cross-tolerance with opioids such as morphine also implicates opioid peptide mechanisms in a physiological or pharmacological response. Also, clonidine has been shown to release immunoreactive β-endorphin from the anterior pituitary (Pettibone and Muellar, 1981a) and to increase plasma levels of this opioid peptide in intact but not hypophysectomized rats (Pettibone and Muellar, 1981b). Because β-endorphin has potent hypotensive properties after systemic administration in anesthetized rats (Lemaire *et al.*, 1978), it was possible that pituitary secretion of this peptide could contribute to the clonidine-induced hypotension.

Methods

Experimental animals. Male SHR of the Okamoto strain (300–400 g) were obtained from St. Thomas' Hospital (London, U.K.). Sex and weight matched Wistar rats were obtained from Charles River Laboratories (London, U.K.). The animals were housed at constant temperature with a fixed light-dark schedule. Hypophysectomized and sham-operated Wistar rats (160–200 g at the time of operation) were obtained 1 week postoperatively from Charles River Laboratories and used immediately. They had received food, water, rock salt and lump sugar *ad libitum*. Morphine-dependence was induced by the method of Wei *et al.* (1973) and involved s.c. implantation of a pellet containing 75 mg of morphine on day 1 and two such pellets on day 2 under light halothane anesthesia. The animals were used on day 4 or 5.

Experimental procedures. Animals were anesthetized with Inactin (100–110 mg/kg i.p.). A cannula was placed in the carotid artery for recording blood pressure and for blood sampling and the exterior jugular vein was cannulated for drug administration. The trachea also was cannulated to facilitate respiration. Body temperature was maintained with a heating blanket. Statham or Bell and Howell pressure transducers were connected to the arterial cannulas and pulsatile blood pressure was recorded continuously on a Grass polygraph (model 79D). Heart rate and mean arterial pressure were determined at intervals from the pulse pressure.

Previous experiments indicated that the maximal fall in blood pressure and heart rate produced by clonidine occurred within 10 min and lasted for approximately 60 min (Conway and Jarrott, 1980) and that the maximal fall in plasma norepinephrine concentrations occurred within 5 min (unpublished results). This corresponds with estimates of the elimination half-life of clonidine in rats of approximately 60 min after a distribution half-life of less than 5 min (Conway and Jarrott, 1982). Under these pharmacokinetic conditions it is possible to obtain cumulative dose-response curves and these were constructed according to the following schedule: at 0 min isotonic saline (1 ml/kg) or naloxone (2 mg/kg) was administered. Doses of clonidine of 0.625, 0.625, 1.25, 2.5, 5 and 10 μg/kg were then administered successively at 10, 20, 30, 40, 50 and 60 min, respectively. Blood samples (0.4 ml) were taken 5 min after drug injection and mean blood pressure and heart rate were determined 10 min after.

Clonidine also produces an initial dose-dependent elevation in blood pressure which is less than 4 min in duration (Conway and Jarrott, 1980) and corresponds to the rapid distribution phase of the drug. This pressor response does not influence the prolonged fall in blood pressure after clonidine administration and has not been analyzed further.

The dose of naloxone selected (2 mg/kg) is identical to that used by Farsang *et al.* (1980) to antagonize the responses of clonidine in SHR and twice that used by Petty and de Jong (1982) to inhibit the cardiovascular effects of centrally administered α-methylnorepinephrine. The half-life of naloxone in rats is similar to that of clonidine (Misra *et al.*, 1976; Tepperman *et al.*, 1983) and in a previous study in the same rat strain naloxone (0.8 mg/kg i.a.) was effective in blocking the actions of exogenously applied opioids for over 60 min (Conway *et al.*, 1983).

Blood was not sampled after injections of 5 and 10 μg/kg of clonidine as preliminary studies had shown that no further fall in plasma norepinephrine occurred. Total blood volume sampled was 2 ml, approximately 15% of the circulating volume, but this was replaced with the drug injection volume (1 ml/kg) plus additional isotonic saline. In a series of control experiments in Wistar rats, successive doses of isotonic saline were administered on the same schedule. Plasma was separated from blood samples and stored at −80°C until measurement of plasma norepinephrine levels as described by Brown and Jenner (1981).

Differences were found in the cardiovascular responses to clonidine in SHR and in morphine-dependent rats. Because these could result from altered pharmacodynamic handling of the drug, plasma clonidine levels were determined in rats anesthetized and cannulated according to the above procedures. A dose of 20 μg/kg of clonidine was injected and blood was sampled at 1, 5, 15 and 30 min. Plasma was stored at −20°C until assay for clonidine by radioimmunoassay (Conway and Jarrott, 1980).

Drugs used were clonidine hydrochloride (Boehringer Ingelheim, Germany), naloxone hydrochloride (Du Pont, Wilmington, DE) and Inactin [5-ethyl-5(1-methyl-propyl)-2-thiobarbitone sodium] kindly donated by Dr. Pittman (Byk Gulden, Konstanz, Germany). Morphine tablets were supplied by Dr. S. Hart (Chelsea College, London) and clonidine antibodies by Dr. B. Jarrott (University of Melbourne, Melbourne, Australia).

Statistical procedures. All data are expressed as means ± S.E.M. Mean blood pressure and heart rate measurements for the dose-response curves have been represented as percentage of decreases compared to time zero.

The statistical procedure used to analyze the dose-response curves has been described by Wallenstein *et al.* (1980). In essence, the dose-response data obtained from each animal are summarized by one or more parameters. These parameters are subsequently grouped and comparisons made between the groups. The parameters that were calculated from the data obtained in each rat were: 1) the slope of the dose-response curve. This was determined by least-squares linear regression analysis of the first 4 points on the curve as these points best described the linear portion of the curve. Comparisons of the slopes between groups is informative of differences in trends (Wallenstein *et al.*, 1980) and in the present study indicate differences in the decrease in blood pressure per unit dose. In two cases heart rat data could not be described by a straight line so this data was omitted from the comparisons between groups. 2) The mean of the responses represented as $\sum$ response/n; n = the number of responses. However, to be consistent with the slope calculations only the first 4 points were used. In the present study this parameter provides an index of the magnitude of the decrease in blood pressure, heart rate or plasma norepinephrine produced by clonidine over a set range of doses.

For the decrease in plasma norepinephrine levels only the mean response ($\sum$ response/n) was calculated.

Comparisons of these parameters between groups were made either by unpaired t test or two-way analysis of variance. In the experiments in which plasma concentrations of clonidine were determined, the area under the plasma concentration *vs.* time curve was calculated and compared between groups by two-way analysis of variance.

Results

Effect of naloxone on clonidine responses in normotensive rats and SHR. Mean arterial blood pressure in the SHR was 190 ± 4.5 mm Hg (n = 15) compared to 116 ± 3.7 mm Hg (n = 15) in normotensive rats and heart rates were 357 ± 9.5 bpm (n = 15) and 360 ± 5.8 bpm (n = 15), respectively. Clonidine produced a dose-dependent fall in blood pressure and heart rate in both groups of rats and these responses were virtually unchanged after administration of naloxone (2 mg/kg) (fig. 1). Analysis of the dose-response curves indicated that naloxone had no significant effect on either the slopes or $\sum$ responses/n (tables 1 and 2). However, there was a highly significant increase in both slope and $\sum$ response/n of the blood pressure response curves in SHR compared to normotensive

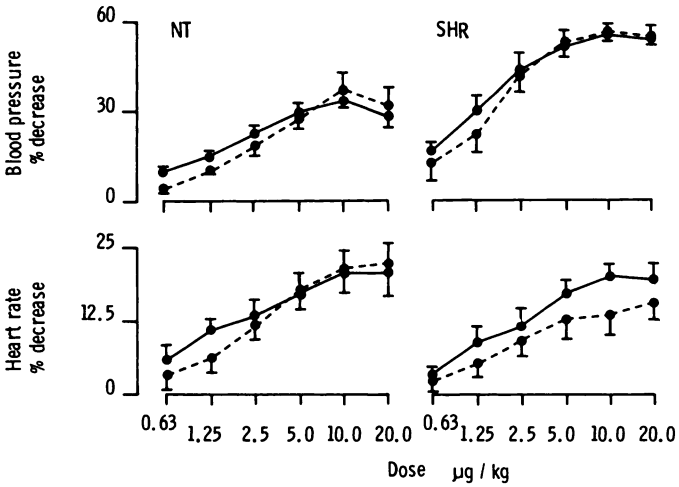

Fig. 1. Effects of clonidine on mean arterial blood pressure and heart rate after pretreament with isotonic saline (——) or naloxone (2 mg/kg i.v.) (– – –) in anesthetized normotensive rats (NT) or SHR. Blood pressure and heart rate changes were determined 10 min after increasing doses of clonidine i.v.

TABLE 1

Summary of blood pressure dose-response curves to clonidine in anesthetized rats

	Slope	$\sum$ response/*n*	*n*
NT[a]	23.6 ± 2.62	19.0 ± 2.62	8
NT + NAL	25.7 ± 4.02	14.8 ± 2.23	7
SHR	40.4 ± 4.58[b]	36.0 ± 3.58[b]	8
SHR + NAL	47.2 ± 6.61	32.5 ± 4.38	7
NT-MPD	30.8 ± 5.06[c]	35.3 ± 3.20[c]	8
SHR-MPD	42.2 ± 4.70	44.4 ± 1.78	7

[a] NT, normotensive; NAL, naloxone (2 mg/kg); MPD, morphine-dependent.

[b] Two-way analysis of variance of rat strains and pretreatment with naloxone indicated no effect of naloxone on either slope (*F* ratio = 0.96, *F* probability = .34) or $\sum$ response/*n* (*F* ratio = 1.37, *F* probability = .25), but a highly significant difference in both slope (*F* ratio = 17.34, *F* probability = .0003) and $\sum$ response/*n* (*F* ratio = 27.65, *F* probability = .00002) between SHR and normotensive rats.

[c] Two-way analysis of variance of rat strains and presence or absence of morphine dependence again indicated a similar separation on strain (statistics not shown). In morphine-dependent rats the slopes were not different (*F* ratio = 1.17, *F* probability = .29) but there was a significant separation in the $\sum$ response/*n* (*F* ratio = 17.92, *F* probability = .00024).

TABLE 2

Summary of heart rate dose-response curves to clonidine in anesthetized rats

	Slope	$\sum$ Response/*n*	*n*
NT[a]	13.3 ± 2.11	11.4 ± 2.95	8
NT + NAL	16.3 ± 2.25	9.5 ± 2.15	7
SHR	14.8 ± 2.26[b]	10.0 ± 1.99[b]	8
SHR-NAL	12.7 ± 2.97	6.5 ± 2.50	6
NT-MPD	22.8 ± 4.40[c]	20.7 ± 2.27[c]	7
SHR-MPD	27.2 ± 2.99	18.3 ± 2.74	7

[a] NT, normotensive; NAL, naloxone (2 mg/kg); MPD, morpine-dependent.

[b] Two-way analysis of variance of rat strains and pretreatment with naloxone indicated no effect of naloxone on either slope (*F* ratio = 0.05, *F* probability = .83) or $\sum$ response/*n* (*F* ratio = 1.13, *F* probability = .30) and also no difference in slope (*F* ratio = 0.09, *F* probability = .77) or $\sum$ response/*n* (*F* ratio = 0.71, *F* probability = .41) between SHR and normotensive rats.

[c] Two-way analysis of variance of rat strains and presence or absence of morphine-dependence again indicated no separation on strain (statistics not shown). However, in rats made morphine-dependent there was a highly significant separation on both slope (*F* ratio = 13.57, *F* probability = .0011) and $\sum$ response/*n* (*F* ratio = 12.19, *F* probability = .0017).

rats. This is illustrated by the steeper dose-response curve and the higher plateau observed in SHR (fig. 1). Heart rate responses were not different in the two groups (table 2).

Clonidine also produced a dose-dependent fall in plasma norepinephrine concentrations. This effect was variable at the lower doses of clonidine and $\sum$ response/*n* varied quite markedly within rat groups as indicated by the large S.E.M. (table 3). Hence, although naloxone reduced $\sum$ response/*n* in normotensive animals, this effect was not significant. Control plasma norepinephrine concentrations were significantly elevated in SHR compared to normotensive rats; however, the percentage of reduction in these levels produced by clonidine ($\sum$ response/*n*) was identical in the two groups (table 3).

In normotensive rats successive injections of isotonic saline produced no significant effects on plasma norepinephrine concentrations (table 3). Blood pressure was reduced slightly by a maximum of 9.1% and heart rate decreased by 3.1% which was not significant (data not shown).

Effects of clonidine in normotensive rats and SHR made morphine-dependent. In figure 2 the blood pressure and heart rate responses to successive doses of clonidine are shown in morphine-dependent normotensive rats and SHR and their corresponding untreated controls. Preinjection levels of blood pressure and heart rate were unchanged in the normotensive rats made morphine-dependent [116 ± 3.7 mm Hg (*n* = 15) *cf.*, 110 ± 4.8 mm Hg (*n* = 8) and 357 ± 10 bpm of 351 ± 10 bpm]. In SHR, blood pressure was not significantly different in the group made morphine-dependent [168 ± 11 mm Hg, *cf.* (*n* = 7), 190 ± 5 mm Hg (*n* = 15)]; however, heart rate was slightly reduced (319 ± 13.6 bpm, *cf.*, 360 ± 5.8 bpm, $P < .05$).

Morphine dependence produced an upward shift in the blood pressure response curves illustrated by no change of slopes but a highly significant increase in the $\sum$ response/*n* parameter of the curves (table 1). Thus, in morphine-dependent rats of both

TABLE 3

Effect of clonidine administration on plasma norepinephrine levels (nanograms per milliliter) in anesthetized rats

	Control	Clonidine dose (µg/kg)				$\sum$ Response/*n*
		0.625	1.25	2.5	5.0	
NT[a]	0.29[b] ±0.04	0.24 ±0.04	0.18 ±0.01	0.17 ±0.01	0.13 ±0.01	32.9[c] ±8.6
NT + NAL	0.24 ±0.03	0.26 ±0.02	0.22 ±0.03	0.22 ±0.03	0.15 ±0.01	6.8 ±11.2
SHR	0.33 ±0.06	0.27 ±0.04	0.24 ±0.03	0.19 ±0.01	0.15 ±0.01	31.5 ±7.8
SHR + NAL	0.46 ±0.03	0.36 ±0.05	0.34 ±0.04	0.27 ±0.02	0.23 ±0.04	34.9 ±6.0
NT-MPD	0.21 ±0.02	0.16 ±0.01	0.18 ±0.04	0.12 ±0.01	0.08 ±0.01	33.7 ±6.6
SHR-MPD	0.44 ±0.04	0.43 ±0.07	0.35 ±0.06	0.30 ±0.07	0.19 ±0.04	30.4 ±6.0
HYP	0.38 ±0.04	0.32 ±0.07	0.25 ±0.05	0.26 ±0.04	0.16 ±0.02	33.5 ±7.1
NT-placebo[d]	0.20 ±0.03	0.23 ±0.04	0.26 ±0.04	0.24 ±0.03	0.24 ±0.05	

[a] NT, normotensive; NAL, naloxone (2 mg/kg); MPD, morphine-dependent; HYP, hypophysectomized.

[b] Control norepinephrine concentrations: two-way analysis of variance between NT and SHR indicated a significant difference between NT and SHR (*F* ratio = 3.60, *F* probability = .041) but not between treatments (*F* ratio = 0.64, *F* probability = .53).

[c] $\sum$ response/*n*: two-way analysis of variance indicated no significant effect of either strain (*F* ratio = 1.51, *F* probability = .24) or pretreatment (*F* ratio = 0.67, *F* probability = .52).

[d] NT-placebo, successive administration of isotonic saline instead of clonidine.

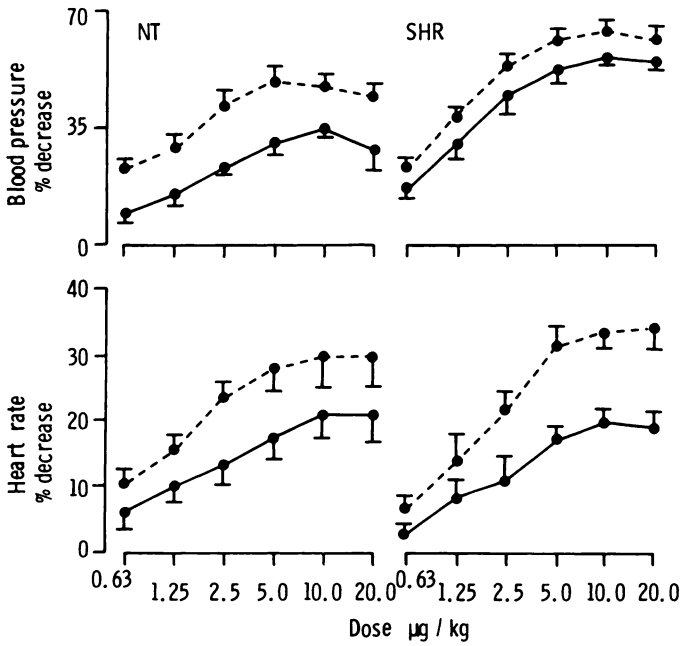

Fig. 2. Effects of clonidine on mean arterial blood pressure and heart rate in untreated (——) and morphine-dependent (- - -) normotensive rats (NT) and SHR. Blood pressure and heart rate changes were determined 10 min after increasing doses of clonidine i.v.

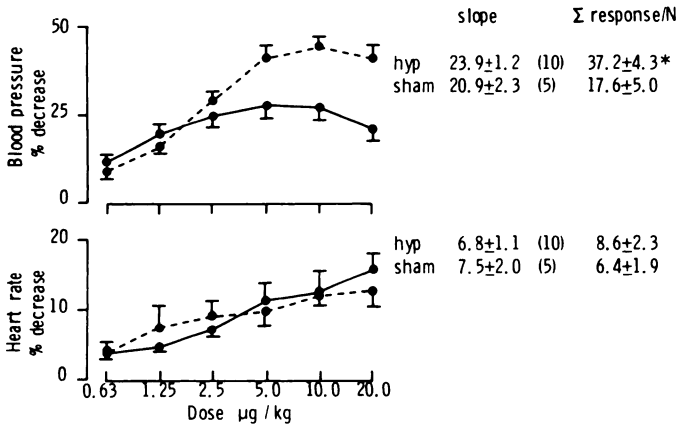

Fig. 3. Effects of clonidine on mean arterial blood pressure and heart rate in sham-operated (——) and hypophysectomized (- - -) anesthetized normotensive rats. Blood pressure and heart rate changes were determined 10 min after increasing doses of clonidine i.v. Slope and response/n of the dose-response curves were calculated as described under "Methods" and compared by unpaired t test. * $P < .05$.

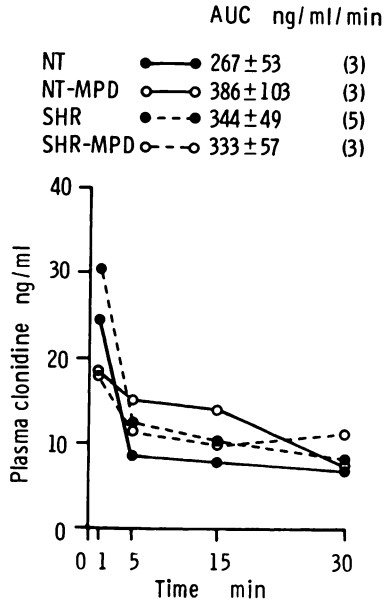

Fig. 4. Plasma clonidine levels after clonidine (20 µg/kg i.v.) in anesthetized rats. NT, normotensive rats; MPD, morphine-dependent rats. Two-way analysis of variance of areas under curve (AUC) indicated no significant differences in rat strains (F ratio = 0.08, F probability = .75) or in pretreatment (F ratio = 0.49, F probability = .50).

strains there is a significant enhancement of the blood pressure response. There was also a significant enhancement of the heart rate response to clonidine produced by morphine dependence. This included not only an increase in $\sum$ response/n but also a significantly steeper slope (table 2).

The effect of clonidine on plasma norepinephrine concentrations was similar in each group of rats (table 3).

Effects of clonidine in hypophysectomized rats. Preinjection blood pressure in hypophysectomized rats was not significantly different from that in sham-operated controls [92 ± 5 mm Hg (n = 10), *cf.*, 101 ± 8 mm Hg (n = 5)]; however, heart rate levels were approximately 30% lower (279 ± 12 bpm, *cf.*, 384 ± 17 bpm, $P < .0001$). Heart rate responses to successive increasing doses of clonidine were virtually identical in the two groups (fig. 3). Although the slopes of the blood pressure response curves were similar, there was a greater maximal response to clonidine in the hypophysectomized rats reflected in the significant increase in $\sum$ response/n of this group compared to sham-operated rats (fig. 3).

Plasma norepinephrine concentrations were not determined in sham-operated rats; however, the effect of clonidine on plasma norepinephrine concentrations in hypophysectomized rats compared to normotensive rats was not significantly different (table 3).

Plasma clonidine levels in normotensive rats, SHR and corresponding morphine-dependent groups. The disappearance of clonidine in all four groups of rats showed approximately the same time-course (fig. 4). The area under the plasma concentration curves was compared using two-way analysis of variance and there was no significant difference between either normotensive rats and SHR or between nontreated- and morphine-dependent rats (fig. 4).

Discussion

In the studies presented here naloxone at an effective dose (see "Methods") did not modify the clonidine-induced decreases in blood pressure, heart rate and plasma norepinephrine concentrations in anesthetized normotensive rats and SHR. Furthermore, rather than there being any inhibition of these responses in the corresponding morphine-dependent rat groups or in hypophysectomized animals (see introductory section), there was an enhancement of the hypotensive and bradycardic effects even though clonidine levels were similar. These results argue against there being any role of endogenous opioid peptides in mediating the cardiovascular responses to clonidine and support previous findings in anesthetized rats (Gomes *et al.*, 1976), cats (Shropshire and Wendt, 1983) and in humans (Watkins *et al.*, 1980; Rogers and Cubeddu, 1983). In additional studies in anesthetized cats there was no evidence that nalox-

one administered either into a lateral cerebral ventricle, the cisterna magna or the peripheral circulation antagonized the hypotension produced by clonidine (W. Feldberg and E. L. Conway, unpublished observations).

Farsang *et al.* (1980) have reported that naloxone does inhibit the clonidine-induced hypotension and bradycardia in conscious and anesthetized SHR but not in normotensive rats. The latter finding is in agreement with the present results but those in SHR are different. In their studies in conscious rats the indirect tail-cuff procedure was used to determine blood pressure and heart rate. This method has been shown to produce a greater elevation in blood pressure and circulating catecholamines in SHR than in normotensive rats when compared to direct recordings in resting animals (Chiueh and Kopin, 1978). This hyper-responsiveness to stress may explain the discrepancy in results. Stress-induced analgesia is a well documented phenomenon (Madden *et al.*, 1977; Amir *et al.*, 1980; Grau *et al.*, 1981) and has been associated with release of opioid peptides from the pituitary (Lim and Funder, 1983) and the adrenal medulla (Lewis *et al.*, 1982). If these circulating opioid peptides have cardiovascular depressant effects as has been demonstrated in pharmacological studies (Lemaire *et al.*, 1978; Moore and Dowling, 1980; Sitsen *et al.*, 1982), then effects of naloxone on clonidine responses observed by Farsang *et al.* (1980) may represent a more complex interaction than has been supposed. Also, reversal of any stress-induced analgesia might complicate the cardiovascular responses to clonidine in conscious animals.

The different results in anesthetized animals are difficult to explain. Farsang *et al.* (1980) used rats lightly anesthetized with pentobarbital. In the present study Inactin was used which, although also a barbiturate, produces less reduction in blood pressure and a more stable anesthesia for cardiovascular studies than does pentobarbital (unpublished observations). Anesthetic interactions may therefore explain the different results.

Enhanced cardiovascular responses to clonidine in SHR compared to other strains of rats have been documented previously (Dadkar *et al.*, 1979; Farsang *et al.*, 1980); however, the effect of morphine dependence in enhancing responses in both groups of rats was surprising. Chronic morphine administration in rats decreases sensitivity to the inhibitory effect of morphine on stimulation-induced contractions in isolated vasa deferentia, whereas there is concomitant development of supersensitivity to norepinephrine (Rae and de Moraes, 1983). Moreover, chronic morphine treatment increases the number of *alpha*-2 adrenoceptor binding sites in rat brain (Hamburg and Tallman, 1981). Because clonidine acts *via alpha*-2 adrenoceptors to produce a fall in blood pressure (see introductory section), the present findings lend support to this observation and suggest that there may also be an increase in the number of functional *alpha*-2 adrenoceptors in the brain. Opioid substances decrease catecholamine release *in vitro* (Gaddis and Dixon, 1982; Jones and Marchbanks, 1982; Saiani and Guidotti, 1982) and can also reduce norepinephrine turnover in brain (Gomes *et al.*, 1976; Fuxe *et al.*, 1979) and decrease firing rates in the locus ceruleus (Aghajanian, 1978). Thus, a prolonged suppression of noradrenergic activity may explain the increase in *alpha*-2 adrenoceptors.

Although previous studies had implicated central opioid peptides as potential contributors to the hypotensive action of clonidine (Kunos *et al.*, 1981), it was possible that pituitary release of β-endorphin might also play a role (see introductory section). We found no evidence of this but rather an unexpected increase in responsiveness of blood pressure in hypophysectomized compared to sham-operated rats. Enhanced hypotensive effects of clonidine have been reported in sodium-depleted rats (Pals, 1975); however, this is unlikely to explain the present results as rats were maintained on salt before the experiments. Further study will be required to determine which pituitary factors influence the altered response to clonidine.

Until recently the centrally mediated fall in blood pressure produced by clonidine has been attributed to a decrease in sympathetic nerve activity (Klupp *et al.*, 1970; Loew and Waite, 1974; Tangri *et al.*, 1977). However, there are discrepancies between the time course of clonidine-induced hypotension and reduced sympathetic nerve activity (Schmitt, 1975; Laubie *et al.*, 1976) and between the hypotension and the reduction in circulating norepinephrine concentrations (Louis *et al.*, 1983). In the present studies, despite significant differences in the cardiovascular responses to clonidine, the dose-dependent reduction in plasma norepinephrine concentrations was not significantly different in any of the groups. These results support the possibility that a factor in addition to a reduction in sympathetic nerve activity may be operating in clonidine-induced hypotension. However, they do not implicate endogenous opioid peptides as this factor.

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