The cardiovascular actions of clonidine and neuropeptide-Y in the ventrolateral medulla of the rat

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- 1 The cardiovascular responses to neuropeptide-Y (NPY) (25 and 50 pmol) and clonidine (10 and 20 nmol) were examined following microinjection into the rostral ventrolateral medulla (RVLM) and the caudal ventrolateral medulla (CVLM). Mean arterial pressure (MAP) and heart rate (HR) were measured in anaesthetized rats, pre- and post-injection.
- 2 The α_2 -adrenoceptor agonist clonidine (10 and 20 nmol) reduced MAP and HR significantly when microinjected into the CVLM and RVLM.
- 3 NPY (25 and 50 pmol) microinjected into the CVLM decreased MAP and HR. However, in the RVLM neither dose had a significant cardiovascular effect.
- 4 The possibility of a functional interaction between the adrenergic system and NPY was examined by co-administration of clonidine and NPY in doses that gave submaximal blood pressure responses. In the CVLM this produced hypotension and bradycardia which was similar in magnitude to the sum of their individual responses, indicating that in this area their actions appear to be independent.
- 5 In the RVLM, where NPY has no significant cardiovascular effects, co-administration with clonidine, did not alter the response to clonidine.
- 6 It appears that in the areas investigated, there is no functional interaction between NPY and clonidine.

Introduction

The ventrolateral medulla (VLM) is now considered to be a major centre in the tonic and reflex control of blood pressure (Ciriello *et al.*, 1986). On the basis of neuronal content and different actions on blood pressure this area can be separated into two regions, the rostral ventrolateral medulla (RVLM) and the caudal ventrolateral medulla (CVLM).

The RVLM includes the CI group of adrenaline-containing neurones which project to the intermediolateral cell column of the spinal cord, where the preganglionic sympathetic neurones are situated (Hokfelt *et al.*, 1974; Ross *et al.*, 1981). Electrical or chemical stimulation of these CI neurones elevates mean arterial pressure (MAP) while neuronal blockade lowers MAP to similar levels reached by spinal cord transection, thus suggesting a tonic influence (Ross *et al.*, 1984; Guertzenstein *et al.*, 1978; Routledge & Marsden, 1987). In addition a series of lesioning experiments by Granata *et al.* (1983; 1985a) has shown that this region is also involved in the

vasodepressor baroreflex response via an inhibitory projection from the nucleus tractus solitarius.

Caudal to this region are the noradrenergic AI neurones (Dahlstrom *et al.*, 1964). In contrast to the CI group, these neurones when activated elicit a reduction in MAP and when inhibited an increase (West *et al.*, 1981; Blessing *et al.*, 1982). It has been suggested that a component of this response is due to a reduction in sympathetic nerve activity possibly via an indirect inhibitory input to the CI region (Willette *et al.*, 1984; Granata *et al.*, 1985b; Dampney *et al.*, 1987).

Further characterization of these areas through immunohistochemical studies has revealed that neuropeptide-Y like immunoreactivity (NPY-IR) co-exists with noradrenaline and adrenaline in most of the cell bodies and some terminals of the AI/CI region respectively (Everitt *et al.*, 1984; Chronwall *et al.*, 1985). NPY, a 36 amino acid peptide and member of the pancreatic polypeptide family, was

Figure 1 Effect of 10 nmol (□) and 20 nmol (■) clonidine microinjections into the caudal ventrolateral medulla (CVLM) on (a) mean arterial pressure (MAP) and (b) heart rate (HR). Points represent mean changes compared to saline control (○); vertical bars show s.e.mean. Pre-injection values $\pm$ s.e.mean are as follows; saline ($n = 6$), MAP = 96.13 ± 2.57 , HR = 369 ± 14.1 ; 10 nmol clonidine ($n = 10$), MAP = 96.8 ± 2.50 , HR = 373 ± 8.57 ; 20 nmol clonidine ($n = 5$), MAP = 94.4 ± 1.69 , HR = 339 ± 13.27 . *Significant differences from saline control value ($P < 0.05$).

originally isolated and characterized in porcine brain by Tatemoto *et al.* (1982). A functional role for NPY in the medulla was supported by the observation that intracisternally administered NPY in rats, results in hypotension and bradycardia (Fuxe *et al.*, 1983; Macrae & Reid, 1988). In addition, modulation of the hypotensive responses to NPY and adrenaline given separately, has been reported after intracisternal coadministration (Harfstrand *et al.*, 1984). This may be a result of a change in receptor number or an interaction at the level of the receptor coupling or post receptor events. (Agnati *et al.*, 1983a; Fuxe *et al.*, 1984).

The aim of the present study was to determine the haemodynamic effects of NPY and the α_2 -adrenoceptor agonist clonidine following micro-

injection into the CVLM and RVLM, two important areas of cardiovascular regulation. Furthermore we examined the possibility of a functional interaction between NPY and α_2 -adrenoceptors in these regions.

Methods

Experiments were carried out on male, 250–320 g Wistar-Kyoto rats (Harlan Olac, Bicester, U.K.) anaesthetized with a mixture of urethane (1 g kg^{-1} , i.p.) and α -chloralose suspension (60 mg kg^{-1} , i.p.). The rats were placed in a David Kopf stereotaxic frame. For the CVLM the incisor bar was set at 5 mm above the intra-aural line (IAL) and the co-ordinates were as follows: 4.5 mm caudal to the IAL; 1.7 mm lateral to the midline and 9.9 mm ventral to the cerebellar surface. To locate the RVLM the incisor bar was set at 5 mm below the IAL and the co-ordinates were: 3.5 mm rostral to the IAL; 2.4 mm lateral to the midline and 8.5 mm ventral to the cerebellar surface (Paxinos & Watson 1986). A small craniotomy was performed and unilateral microinjections of drug or vehicle (0.9% saline), were delivered via a 30-gauge needle with a $1 \mu\text{l}$ microsyringe (Hamilton). All injections were made in a total volume of $0.1 \mu\text{l}$ over a 20 s interval. MAP and heart rate (HR) were measured via a heparinised catheter positioned in the abdominal aorta, connected to a Gould pressure transducer and linked to a Grass polygraph, with a tachograph for direct HR recording. The catheter was surgically implanted the previous day under a combination of sodium pentobarbitone (60 mg kg^{-1}) and chloral-hydrate (100 mg kg^{-1}) anaesthesia and used to monitor MAP and HR for 30 min pre- and 120 min post-injection. Temperature was maintained at 37°C with a homoeothermic blanket. At the end of each experiment the animal was killed by an overdose of sodium pentobarbitone. The brain was then fixed by transcardiac perfusion with FAGLU (formaldehyde 4% and glutaraldehyde 0.5% in 0.1 M sodium phosphate buffer pH 7.0), excised and $50 \mu\text{m}$ sections were cut and mounted for verification of injection site. Experiments where the injection site was not within the AI or CI region were excluded from the study. Statistical analysis was carried out on the changes in MAP and HR by use of ANOVA with Bonferoni test for multiple comparisons. All values were expressed as mean $\pm$ s.e.mean.

Drugs

Drugs used were porcine neuropeptide-Y (Cambridge Research Biochemicals) and clonidine

Figure 2 Effect of 25 pmol (Δ) and 50 pmol ($\blacktriangle$) neuropeptide-Y (NPY) microinjections into the caudal ventrolateral medulla (CVLM) on (a) mean arterial pressure (MAP) and (b) heart rate (HR). Points represent mean changes compared to saline control ($\circ$); vertical bars show s.e.mean. Pre-injection values $\pm$ s.e.mean are as follows: saline ($n = 6$), MAP = 96.13 ± 2.57 , HR = 369 ± 14.1 ; 25 pmol NPY ($n = 9$), MAP = 101 ± 1.91 , HR = 388 ± 11.3 ; 50 pmol NPY ($n = 9$), MAP = 102 ± 2.67 , HR = 368 ± 9.17 . *Significant differences from saline control value ($P < 0.05$).

hydrochloride (Sigma Chemicals Co.). Drugs were dissolved in sterile saline, aliquoted and stored at -70°C .

Results

No significant haemodynamic response was observed after injection of $0.1 \mu\text{l}$ of vehicle (0.9% saline) into the CVLM or the RVLM. Microinjection of the peptide or clonidine into nearby control areas lateral or dorsal to the AI or CI area produced no significant haemodynamic response

Effects of clonidine and neuropeptide-Y microinjections into the CVLM

Clonidine Microinjections of clonidine (10 and 20 nmol) in $0.1 \mu\text{l}$ elicited a significant dose-related fall in MAP when microinjected into the CVLM. This effect reached a nadir at 45 min (-21.0 ± 4.59 mmHg; -40.8 ± 6.53 mmHg respectively) and

Figure 3 Effect of 10 nmol ($\square$) and 20 nmol ($\blacksquare$) clonidine microinjections into the rostral ventrolateral medulla (RVLM) on (a) mean arterial pressure (MAP) and (b) heart rate (HR). Points represent mean changes compared to saline control ($\circ$); vertical bars show s.e.mean. Pre-injection values $\pm$ s.e.mean are as follows: saline ($n = 6$), MAP = 89.17 ± 3.27 , HR = 368.3 ± 18.3 ; 10 nmol clonidine ($n = 8$), MAP = 97.25 ± 1.86 , HR = 375 ± 15.9 ; 20 nmol clonidine ($n = 5$), MAP = 88 ± 6.04 , HR = 347 ± 29.14 . *Significant differences from saline control value ($P < 0.05$).

the responses took 2–3 h to return to pre-injection levels. The maximum reduction in HR, was similar for both doses of clonidine (-131 ± 17.42 bpm; -121 ± 22.11 bpm respectively). As shown in Figure 1, these falls were also sustained for 2–3 h.

Neuropeptide-Y The results after microinjection of NPY (25 and 50 pmol) into the CVLM are summarized in Figure 2. Both doses of NPY microinjected into this area produced a fall in MAP which reached a maximum at 45 min (-7.44 ± 2.16 mmHg; -12.33 ± 3.01 mmHg respectively) and returned to control saline values after 1 h. In addition the bradycardia produced by NPY (25 and 50 pmol) resembled that after clonidine in this area. Both con-

centrations of NPY produced similar falls in HR with the nadir reached at 45 min (-91.1 ± 21.45 bpm; -94.4 ± 28.14 bpm respectively) and sustained for 1 h.

Effects of clonidine and neuropeptide-Y microinjections into the RVLM

Clonidine A profound fall in MAP and HR was observed after clonidine (10 and 20 nmol) was injected into the RVLM. This was similar to the response of clonidine in the CVLM. The results are shown in Figure 3. The maximum reduction in MAP (-21.63 ± 5.85 mmHg (10 nmol) and -36.0 ± 3.55 mmHg (20 nmol)) occurred at 30 min with the hypotension lasting 2–3 h at the highest concentration. However in this area, unlike the caudal region, the bradycardia induced by clonidine was dose-related, reaching a nadir of -54.6 ± 12.17 bpm (10 nmol) and -110 ± 16.2 bpm (20 nmol) at 30 min post-injection.

Neuropeptide-Y In the RVLM neither concentration of NPY studied (25 and 50 pmol) influenced blood pressure or heart rate and results were not significantly different from the saline control (Figure 4). Although clonidine in the RVLM produced a dose-

Figure 4 Effect of 25 pmol (Δ) and 50 pmol (▲) neuropeptide-Y (NPY) microinjections into the rostral ventrolateral medulla (RVLM) on (a) mean arterial pressure (MAP) and (b) heart rate (HR). Points represent mean changes compared to saline control (○); vertical bars show s.e.mean. Pre-injection values $\pm$ s.e.mean are as follows; saline ($n=6$), MAP = 89.17 ± 3.27 , HR = 368.83 ± 18.3 ; 25 pmol NPY ($n=7$), MAP = 97.86 ± 4.86 , HR = 382.8 ± 18.35 ; 50 pmol NPY ($n=6$), MAP = 90 ± 2.58 , HR = 360 ± 13.9 . *Significant differences from saline control value ($P < 0.05$).

Figure 5 Co-administration of 25 pmol or 50 pmol neuropeptide-Y (NPY) together with 10 nmol clonidine into the caudal ventrolateral medulla. Columns show change in (a) mean arterial pressure (MAP) and (b) heart rate (HR) (with s.e.mean shown by vertical bars) 30 min post injection. *Significant difference compared to sum of individual responses ($P < 0.05$). n values in columns.

related hypotension and bradycardia similar to its effects in the caudal region, NPY appears to have no significant action on these parameters in this area.

Effects of the co-administration of neuropeptide-Y and clonidine in the rostral and caudal ventrolateral medulla

The results presented for both brain areas represent the responses obtained 30 min after injection of clonidine or NPY injected separately and when co-administered.

CVLM Co-administration of clonidine (10 nmol) and NPY (25 pmol) in the CVLM reduced MAP

Figure 6 Co-administration of 25 pmol neuropeptide-Y (NPY) and 10 nmol clonidine into the rostral ventrolateral medulla. Columns show change in (a) mean arterial pressure (MAP) and (b) heart rate (HR) (with s.e.mean shown by vertical bars) 30 min post injection. *Significant difference compared to sum of individual responses ($P < 0.05$).

by -23.5 ± 3.3 mmHg which was similar to the sum of their individual responses (Figure 5). However the bradycardia was different. The combination was significantly less than the sum of their separate response (-109.0 ± 13.6 bpm). However, when clonidine (10 nmol) was injected with the higher concentration of NPY (50 pmol) into the CVLM both the fall in MAP and HR which resulted could be considered additive (-30 ± 2.36 mmHg; -163.8 ± 8.0 bpm).

RVLM Although in the RVLM, NPY (25 and 50 pmol) alone produced no significant cardiovascular effect, it is possible that it could modulate the response to other agents which have an effect here. Therefore, the cardiovascular effects of NPY (25 pmol) and clonidine (10 nmol) injected together (-16.7 ± 4.6 mmHg; -68.8 ± 20.1 bpm) were investigated (Figure 6). For both blood pressure and heart rate, the response to co-administered clonidine and NPY was not significantly different from clonidine alone (-21.6 ± 5.9 mmHg; -54.6 ± 12.2 bpm). There is therefore no evidence to suggest an interaction between NPY and α_2 -adrenoceptors at this site.

Discussion

In this study further evidence has been presented which supports a vasodepressor role for NPY in the medulla of the rat (Fuxe *et al.*, 1983; Macrae & Reid, 1988). NPY (25 and 50 pmol), when injected by a discrete microinjection technique, into the CVLM of the anaesthetized rat, evoked a dose-related hypotension. This effect may be mediated through the specific NPY receptors which have been demonstrated in the medulla by high affinity binding studies (Harstrand *et al.*, 1986). Although NPY elicited a pronounced fall in heart rate in the CVLM, it was not dose-related. One explanation for this may be the close proximity and possible involvement of the nucleus ambiguus, an area with cardio-inhibitory vagal projections (Ciriello & Calaresu, 1979; Paxinos & Watson, 1986). Macrae & Reid (1988) have shown that the bradycardia elicited by CVLM microinjections of NPY in vagotomized rats, is significantly reduced compared to control rats. It is possible that the mechanisms involved in the regulation of heart rate are more sensitive to NPY than the response to blood pressure. Thus although the blood pressure response to the lower dose of NPY was submaximal, the heart rate response may have been maximal.

The hypotension and bradycardia observed following the injection of clonidine (10 and 20 nmol) into the RVLM and CVLM is consistent with reports by Punnen *et al.* (1987) and previous studies where other regions of the medulla have been investigated (Chan & Koo, 1978; Carter *et al.*, 1985). However, in the CVLM, the bradycardia produced by clonidine was not dose-related. In this respect the response resembled the action of NPY in this area and may again reflect a greater sensitivity in the mechanisms controlling heart rate compared to those controlling blood pressure.

The haemodynamic effects produced by clonidine are thought to involve α_2 -adrenoceptors which are

widely distributed in the medulla. Unnerstal *et al.* (1984) demonstrated that the distribution of these α_2 -adrenoceptors in the CNS, as identified by [^3H]-*p*-aminoclonidine binding, correlates well with the distribution of central noradrenergic and adrenergic neurones.

Some investigators believe that clonidine lowers MAP via an α_2 -presynaptic mechanism which inhibits the release of noradrenaline or adrenaline from the medullary neurones (Scatton *et al.*, 1979; Atkinson *et al.*, 1984). In addition, Bousquet *et al.* (1984) proposed that clonidine may act on 'imidazole preferring sites', which possess a pharmacologically distinct profile from that of α_2 -adrenoceptors. However, local brainstem networks containing γ -aminobutyric acid, acetylcholine or catecholamines can also modify the actions of ventrolateral medullary neurones and may account for the similar actions of clonidine that we observed in both a vasopressor (Cl) and vasodepressor (Al) area (Reis, 1987).

Central immunohistochemical studies show that many of the classical catecholamine neurones also store and probably release biologically active peptides as cotransmitters (Everitt *et al.*, 1984; Hokfelt *et al.*, 1987). Evidence for a functional role of NPY/adrenaline or NPY/noradrenaline coexistence in neurones in cardiovascular centres of the medulla, comes from *in vitro* studies by Agnati *et al.* (1983a,b) and Fuxe *et al.* (1986; 1987) who describe a reciprocal modulation of receptor number and affinity. Moreover, in *in vivo* experiments the fall in MAP and HR after intraventricular NPY was unaltered by α_2 -blockade, suggesting an independent mechanism of action (Harfstrand *et al.*, 1984). However, the

response to intraventricular NPY when coadministered with adrenaline was not additive implying a functional interaction (Harfstrand & Fuxe, 1987). We have employed a method of discrete microinjections which allowed us to study the possibility of *in vivo* interactions in the CVLM and RVLM. In the CVLM the combination of NPY together with clonidine produced a hypotensive effect which was additive. The bradycardia elicited after coadministration of these agents was also additive after combination of clonidine with the highest dose of NPY. In the RVLM however, NPY alone had no effect on either parameter measured and when coadministered with clonidine NPY did not alter the response to the latter. Thus, the two agents did not appear to antagonize the action of each other, as previously claimed (Fuxe *et al.*, 1986). In conclusion, we have demonstrated a fall in blood pressure and heart rate after NPY and clonidine given alone into the caudal ventrolateral medulla and for clonidine alone into the rostral ventrolateral medulla, areas of importance in the tonic and reflex control of blood pressure. However, there was no apparent interaction between clonidine and NPY when coadministered suggesting that, at least in these regions, their effects are independent.

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