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A COMPARISON OF THE CARDIOVASCULAR AND SEDATIVE ACTIONS OF THE α-ADRENOCEPTOR AGONISTS, FLA-136 AND CLONIDINE, IN THE RAT

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1 The cardiovascular and sedative effects of FLA-136 have been compared with those of clonidine after intracerebroventricular (i.c.v.) administration in the rat. The effects of both drugs on pre- and postsynaptic α-adrenoceptors in the periphery have been investigated after intravenous (i.v.) administration in the pithed rat.

2 In the anaesthetized rat, i.c.v. FLA-136 and clonidine produced dose-related hypotension, FLA-136 having three to 30 times less activity than clonidine; both drugs caused concomitant bradycardia. In the conscious rat i.c.v. FLA-136 had less sedative potential than clonidine, in terms of overt sedation assessed visually.

3 Yohimbine reduced the hypotension and bradycardia produced by i.c.v. FLA-136 and clonidine; prazosin and mianserin also antagonized the cardiovascular responses to clonidine, but not those to FLA-136.

4 Chemical sympathectomy by 6-hydroxydopamine (6-OHDA) markedly reduced the cardiovascular effects of FLA-136 but only slightly reduced those of clonidine.

5 Naloxone antagonized the cardiovascular responses to clonidine, but not FLA-136, suggesting a direct or indirect involvement of central opiate receptors in the responses induced by clonidine.

6 Metiamide attenuated the cardiovascular responses to FLA-136 and clonidine, implying a direct or indirect involvement of central histamine (H_2)-receptors in such responses.

7 FLA-136, unlike clonidine, did not stimulate peripheral pre- or postsynaptic α-adrenoceptors in the pithed rat.

8 FLA-136 is a novel centrally-acting hypotensive compound which, unlike clonidine, selectively stimulates central α-autoreceptors (yohimbine-sensitive) in the rat; these autoreceptors may be different from peripheral pre- and postsynaptic α-adrenoceptors. The results suggest that clonidine lowers blood pressure by stimulation of two types of central postsynaptic α-adrenoceptors in the rat, one type being sensitive to yohimbine and the other to prazosin.

Introduction

The hypotensive and sedative actions of clonidine are attributed to stimulation of central α-adrenoceptors (Van Zwieten, 1973; 1975; Kobinger, 1978) although histamine (Karppanen, Paakkari, Paakkari, Huotari & Orma, 1976; Finch, Harvey, Hicks & Owen, 1978; Frisk-Holmberg, 1980) and opiate (Farsang & Kunos, 1979) receptors have also been implicated. In the periphery clonidine stimulates both pre (α_2)- and post (α_1)-synaptic α-adrenoceptors (Hoefke & Kobinger, 1966; Starke, Endo & Taube, 1975; Drew, 1976).

Like clonidine, its congener, FLA-136 (Nebidrazine) lowers blood pressure and heart rate after oral administration to conscious hypertensive rats (Eriksson & Florvall, 1976) and decelerates noradrenaline turnover in rat brain by stimulation of α-adrenoceptors sensitive to yohimbine (Anden & Grabowska, 1977). Unlike clonidine, FLA-136 does not stimulate peripheral or central postsynaptic α-adrenoceptors since this compound neither raises blood pressure nor increases the flexor reflex in the rat (Anden, Gomes, Grabowska, Persson & Trolin, 1977; Anden & Grabowska, 1977). Timmermans, Lam & Van Zwieten (1979) have suggested that in the rat the hypotensive activity and stimulation of peripheral presynaptic α_2-adrenoceptors by FLA-136 is due to the action of a metabolite.

FLA-136 therefore possesses some selectivity in its α-stimulant properties and we have compared, in the anaesthetized rat, the cardiovascular responses to centrally administered FLA-136 and clonidine and investigated the nature of the central α-adrenoceptors and possible other (e.g. histamine and opiate) receptors involved in mediating these re-

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sponses. In the periphery the actions of FLA-136 at pre- and postsynaptic α-adrenoceptors have been compared with those of clonidine after intravenous administration in the pithed rat. In addition, the sedative potential of FLA-136 and clonidine have been compared after central administration to conscious rats. A preliminary account of some of these findings has been presented to the British Pharmacological Society (Hamilton & Longman, 1980).

Methods

Male Sprague-Dawley rats (250–400 g) were used for these studies.

Anaesthetized rats

Rats were anaesthetized with pentobarbitone (75 mg/kg i.p.) and the left jugular vein cannulated for administration of drugs. Changes in diastolic blood pressure were recorded from the left common carotid artery with a Bell & Howell physiological pressure transducer (1 mmHg ≈ 133 Pa). The pulse in the blood pressure signal was used to trigger a Devices instantaneous heart ratemeter. Blood pressure and heart rate recordings were displayed on a Devices chart recorder.

By use of a 'David Kopf' stereotaxic instrument, rats were prepared for intracerebroventricular (i.c.v.) injection of drugs through a cannula (Hayden, Johnson & Maickel, 1966) inserted into the lateral cerebral ventricles via a trephine hole drilled 1 mm lateral and 1 mm posterior to the bregma, and secured by means of dental acrylic cement. All i.c.v. injections were made up in 10 μl vehicle introduced at a depth of 3.5–4.0 mm from the top of the skull. At the end of each experiment correct placement of the i.c.v. cannula was verified by injecting 10 μl trypan blue dye, removing the brain and examining the ventricle, macroscopically, for ventricular staining.

Drug administrations

From the dose-response relationships for the reductions in blood pressure and heart rate produced by the i.c.v. administration of FLA-136 and clonidine in the anaesthetized rat (see Figure 1), a submaximal dose of each agonist was chosen for mechanistic studies. The antagonists selected for these studies have been used by others (see Introduction and Discussion for references) to investigate the hypotensive action of clonidine in animals and were used by us in this context for their known relative specificity for blocking selected receptors.

Antagonists were administered i.c.v. 10 min before FLA-136 or clonidine with the exception of prazosin and 6-hydroxydopamine (6-OHDA) which were given by oral and i.c.v. routes respectively using the following pretreatment schedules.

Prazosin: since prazosin given i.c.v. to anaesthetized rats lowered blood pressure, conscious animals were treated orally with prazosin (1 and 0.5 mg/kg on Day 1, 0.5 mg/kg twice on Days 2 and 3) (Cavero & Roach, 1978) before being anaesthetized on Day 4.

6-OHDA: under methohexitone (45 mg/kg i.p.) anaesthesia, i.c.v. cannulae were implanted in rats according to the procedure described previously. On Days 2, 4 and 6 post-operatively rats received either 250 μg 6-OHDA or 10 μl vehicle and on Day 7 the animals were prepared for recording blood pressure and heart rate under pentobarbitone anaesthesia (Haeusler, Finch & Thoenen, 1972).

Overt sedation and core temperature

Under methohexitone (45 mg/kg i.p.) anaesthesia, i.c.v. cannulae were implanted in rats using the procedure previously described. Drugs or vehicle were administered by the i.c.v. route 4 days post-operatively to groups of 6–12 rats and overt sedation assessed, and core temperature measured, 10 min later. Overt sedation was assessed by placing individual animals in a 50 × 50 cm enclosure and observing for gross differences from control rats. The indices used were: ptosis, lowered body posture, slow gait, depressed response to light pressure between finger and thumb placed on either side of body, depressed response to a rod moving across the visual field, passivity and impaired righting reflex (assessed by the number of times the rat failed to land on 4 feet when dropped from an inverted position). These indices were scored on a 0–4 basis according to severity (Drew, Gower & Marriott, 1979). In our experiments, core temperature was also assessed immediately prior to injection of drug or vehicle. Animals were dosed in random manner with vehicle or drug, the observer not knowing the nature of each animal's treatment.

Indirect measurement of blood pressure and heart rate in conscious rats

Rats were placed in an incubator (32–34°C) for 30–40 min. Systolic blood pressure and heart rate were then measured before, and at intervals after, the oral administration of drug using a tail cuff and strain gauge detector coupled to a W & W 8002 blood pressure recorder.

Stimulation of sympathetic outflow in pithed rats

Rats were injected with atropine (1 mg/kg i.p.) and anaesthetized with halothane/nitrous oxide mixture.

After cannulation of the trachea, the animals were pithed through one orbit with a steel rod (Gillespie & Muir, 1967) and received tubocurarine (1 mg/kg i.v.). Changes in blood pressure and heart rate were recorded as described previously. The pithing rod was used to stimulate electrically the entire sympathetic outflow (Gillespie & Muir, 1967) using square-wave pulses of 0.5 ms duration at supramaximal voltage over the frequency range 0.25–8 Hz for periods of 20 s.

Statistical analysis

Students *t* test for paired data was used for comparisons of core temperature and drug-induced inhibition of pressor responses to electrical stimulation in the pithed rat. In all other experiments data was examined using Student's *t* test for grouped data.

Drugs

The following drugs were used: atropine sulphate (B.D.H.), clonidine hydrochloride (Boeringer Ingelheim), 6-hydroxydopamine hydrobromide (Sigma), metiamide (S.K.F.), methohexitone sodium (Lilly), mianserin hydrochloride (Beecham), naloxone hydrochloride (Endo), nebidrazine (FLA-136; 4 - amino - 3 - (2,6 - dichlorobenzylidenehydrazino) - 1,2,4-triazole hydrochloride) (Hassle), phenylephrine hydrochloride (Koch-Light), piperoxan hydrochloride (May & Baker), prazosin hydrochloride (Pfizer), tubocurarine hydrochloride (Burroughs Wellcome), yohimbine hydrochloride (Sigma).

Doses are expressed as base. For intracerebroventricular administration (10 μl dose volume), drugs were dissolved in sterile saline (0.9% w/v NaCl solution) with the exception of FLA-136 and yohimbine which were dissolved in polyethylene glycol and water respectively and 6-OHDA which was dissolved in nitrogen-bubbled 0.01 N HCl. For oral administration (5 ml/kg dose volume), drugs were suspended in methylcellulose.

Results

Central administration of FLA-136 and clonidine

The effect of intracerebroventricular administration of FLA-136 and clonidine on blood pressure and heart rate in the anaesthetized rat In anaesthetized rats FLA-136 (1–100 μg) and clonidine (0.1–10 μg), both i.c.v., caused dose-related falls in diastolic blood pressure; falls in heart rate were dose-related following clonidine but not FLA-136 (Figure 1). The mean fall in diastolic blood pressure evoked by clonidine (1 μg) was similar in magnitude to that following FLA-136 (10 μg); these submaximal doses were selected for further experiments involving antagonists. The maximum hypotensive response to FLA-136 (10 μg) was attained 10 min after administration with a recovery to pre-dose levels within 30 min whilst the response following clonidine (1 μg) had a longer time course, the maximum effect being reached approximately 12 min after injection, a recovery to pre-dose levels being evident after 50 min.

Effects of antagonists on the hypotension and bradycardia to i.c.v. administered FLA-136 and clonidine in the anaesthetized rat Yohimbine (100 μg i.c.v.) antagonized the cardiovascular effects of FLA-136 (10 μg) (Figure 2); at a lower dose (30 μg), yohimbine caused a smaller reduction in the FLA-136 response. Yohimbine (100 μg i.c.v.) significantly reduced the fall in diastolic blood pressure, and reduced the bradycardia, produced by clonidine (1 μg) (Figure 3).

Piperoxan (100 μg i.c.v.) significantly reduced hypotension and bradycardia evoked by administration of FLA-136 (10 μg) (Figure 2).

In rats pretreated orally with prazosin, the cardiovascular effects of FLA-136 (10 μg) were unaffected (Figure 2) whereas the hypotension was significantly attenuated and bradycardia reduced following clonidine (1 μg) (Figure 3).

Figure 2 shows that mianserin (100 μg i.c.v.) did not affect the falls in diastolic blood pressure and heart rate produced by FLA-136 (10 μg) but significantly reduced the hypotensive response, though not the bradycardia, to clonidine (1 μg) (Figure 3).

Figures 2 and 3 show that metiamide pretreatment (300 μg i.c.v.) significantly inhibited the hypotensive responses to FLA-136 (10 μg) and clonidine (1 μg) and reduced the falls in heart rate.

Naloxone (100 μg i.c.v.) had no effect on the falls in diastolic blood pressure and heart rate following FLA-136 (10 μg) (Figure 2) but this pretreatment did significantly attenuate the hypotension and reduced the bradycardia evoked by clonidine (1 μg) (Figure 3).

Resting levels of diastolic blood pressure and heart rate were not significantly altered following i.c.v. administration of the antagonists alone.

Figures 2 and 3 show respectively that the falls in diastolic blood pressure and heart rate produced by FLA-136 (10 μg), but not by clonidine (1 μg), were significantly reduced following central pretreatment with 6-OHDA. The resting levels of diastolic blood pressure and heart rate were significantly lowered ($P < 0.001$) in 6-OHDA pretreated groups of rats compared to control groups. In the experiments involving FLA-136 administration, the mean resting level of diastolic blood pressure was 100 ± 6.4 mmHg

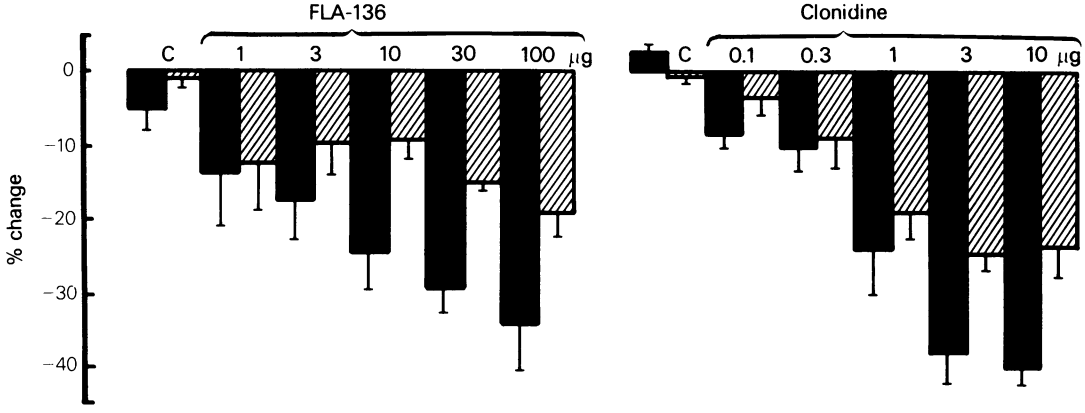

Figure 1 Maximum percentage changes in diastolic blood pressure (solid columns) and heart rate (hatched columns) following intracerebroventricular (i.c.v.) injections of FLA-136, clonidine or vehicle (C) (10 μl dose volume) in 6 anaesthetized rats per group. Vertical bars indicate s.e.mean.

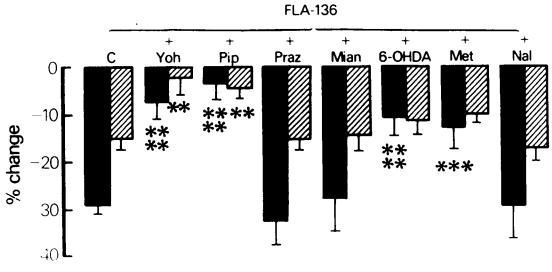

Figure 2 Effects of yohimbine 100 μg (Yoh), piperoxan 100 μg (Pip), mianserin 100 μg (Mian), metiamide 300 μg (Met) and naloxone 100 μg (Nal) on the maximum falls in diastolic blood pressure (solid columns) and heart rate (hatched columns) induced by FLA-136 (10 μg i.c.v.) in 6 anaesthetized rats per group. Prazosin (Praz) and 6-hydroxydopamine (6-OHDA) were administered as oral and i.c.v. pretreatment schedules respectively (see Methods); other antagonists were administered (i.c.v.) (10 μl dose volume), 10 min before FLA-136. Vertical bars indicate s.e.mean. Significant difference from control rats (C) injected with FLA-136 (10 μg i.c.v.) alone indicated by (* $P<0.05$, ** $P<0.02$, *** $P<0.01$, **** $P<0.001$).

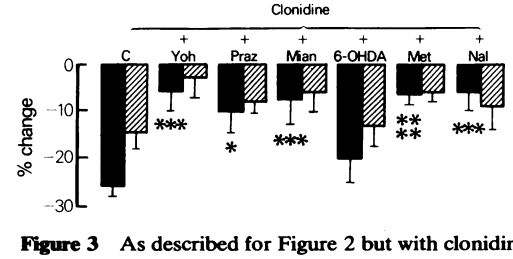

Figure 3 As described for Figure 2 but with clonidine (1 μg i.c.v.) instead of FLA-136.

in 6-OHDA-treated rats compared to a mean resting level of 110 ± 0.2 mmHg in a vehicle pretreated group. In the clonidine investigation the 6-OHDA pretreated rats had resting levels of diastolic blood pressure of 99 ± 4.8 mmHg compared to 105 ± 4.7 mmHg in vehicle pretreated rats.

Sedative and hypothermic actions following i.c.v. administration of FLA-136 and clonidine in conscious rats Figure 4 shows that whereas clonidine (3, 10 and 30 μg) produced dose-related overt sedation and slight hypothermia in conscious rats, the effects of FLA-136 (100, 300 μg and 1 mg) were not clearly dose-related. Although FLA-136, 300 μg, and clonidine, 10 μg, caused similar scores (overt sedation) and hypothermia, a higher dose of FLA-136 diminished, and of clonidine increased, these effects: the maximum responses to FLA-136 were therefore less than those produced by clonidine.

Peripheral administration of FLA-136 and clonidine

Effects of oral administration of FLA-136 on blood pressure and heart rate in conscious rats In conscious normotensive rats FLA-136 (10 and 30 mg/kg; 6 rats/group) caused dose-related reductions in systolic blood pressure without any change in heart rate; no sedative effects were noted during the course of the 6 h experiment. At both doses, the falls in blood pressure were gradual in onset, the maximum effects (−14 ± 2% and −32 ± 3% at 10 and 30 mg/kg respectively) occurring 4 h after dosing. Thereafter blood pressure showed some recovery to pre-dose levels at 6 h, these levels being re-attained at 24 h.

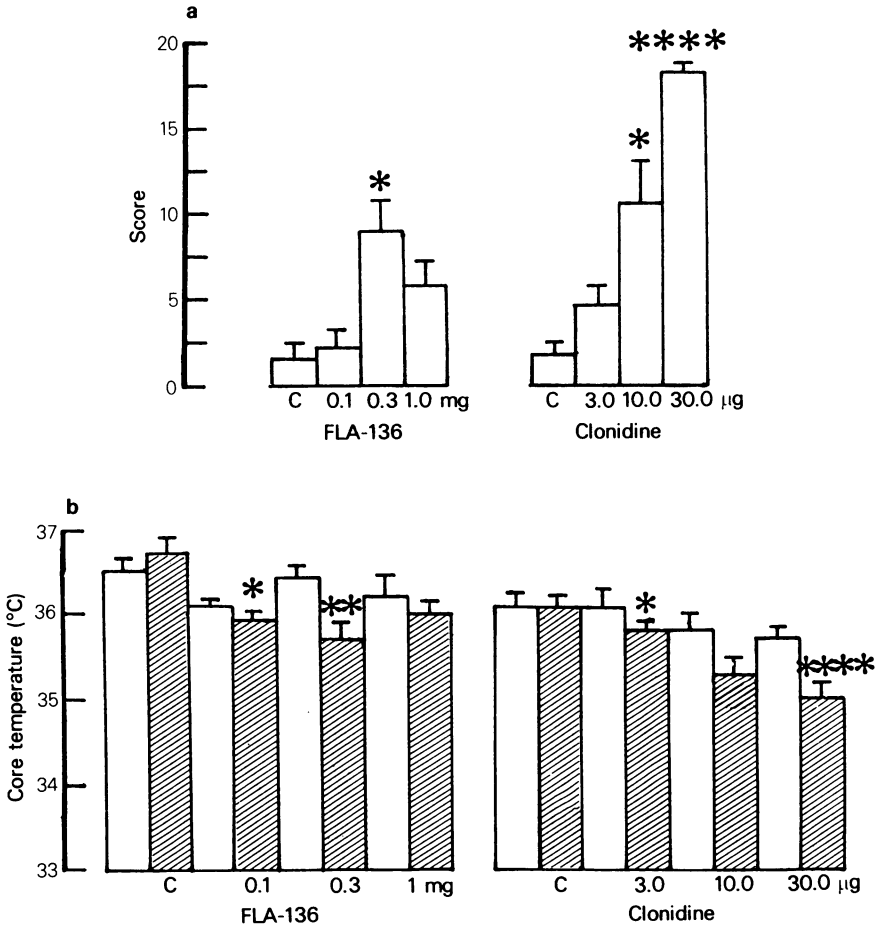

Figure 4 Effects of FLA-136 and clonidine, following (i.c.v.) injection, in causing (a) overt sedation (score), and (b) on core temperature (°C) before (open columns) and after (hatched columns) administration of each drug. Vertical bars indicate s.e.mean. Six rats were used for each dose of clonidine and controls (C); 6–12 rats were used in the FLA-136-treated groups. Significant difference from control rats (C) injected (i.c.v.) with vehicle (group *t* test) or significant difference between pre- and post-dose core temperature (paired *t* test) indicated by: $*P<0.05$; $**P<0.02$; $***P<0.01$; $****P<0.001$.

Effects of intravenous administration of clonidine and FLA-136 on resting blood pressure and heart rate in the anaesthetized rat The doses of FLA-136 (10 and 100 μg) and clonidine (1 and 10 μg) which caused hypotension and bradycardia when administered centrally (Figure 1) were given by the intravenous route in groups of 6 anaesthetized rats. FLA-136 evoked dose-related falls in diastolic blood pressure which were accompanied by dose-dependent bradycardia (Figure 5); these effects were smaller than after the same doses given centrally (Figure 1). The maximum hypotensive response to FLA-136 (10 and 100 μg i.v.) was attained 19 min after administration, signifying a slower rate of onset than when the drug is given by a central route.

Clonidine, at intravenous doses of 1 and 10 μg, caused dose-related pressor effects which preceded the hypotensive responses (Figure 5). Maximum hypotensive responses were obtained 12 and 19 min after the respective intravenous doses indicating a rate of onset slower than that after i.c.v. administration.

Effect of FLA-136 and clonidine on the pressor responses and tachycardia evoked by electrical stimulation of the spinal cord in pithed rats FLA-136 (0.3 mg/kg i.v.) had no effect on the frequency-dependent pressor responses to electrical stimulation

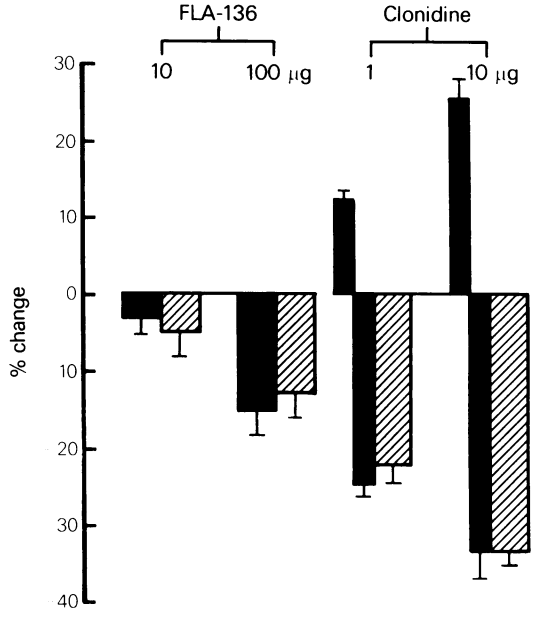

Figure 5 Maximum percentage changes in diastolic blood pressure (solid columns) and heart rate (hatched columns) following intravenous administrations of FLA-136 and clonidine in 6 anaesthetized rats per group. Vertical bars indicate s.e.mean.

in the pithed rat; a slight depression of the neuronally induced tachycardia was seen at the higher frequencies of stimulation (Figure 6). Similar results were obtained with FLA-136 at a higher dose (3 mg/kg i.v.). Oral pretreatment with FLA-136 (10 mg/kg) administered 2.5–3.5 h prior to pithing had no effect on the frequency-dependent pressor responses or tachycardia to electrical stimulation.

Clonidine (30 μg/kg i.v.) inhibited the increases in blood pressure and tachycardia evoked by low frequency (0.25–1 Hz) stimulation of the sympathetic outflow from the spinal cord (Figure 7).

FLA-136 (0.3 and 3 mg/kg i.v.) had no effect on the resting levels of diastolic blood pressure and heart rate of pithed normotensive rats. However, clonidine (30 μg/kg i.v.) evoked an immediate rise (64 ± 11 mmHg; 5 rats) in diastolic blood pressure and concomitant slight bradycardia.

Discussion

In the anaesthetized rat FLA-136, like clonidine, reduces blood pressure and heart rate after central (i.c.v.) administration although FLA-136 possesses three to 30 times less activity than clonidine; a comparison of their relative hypotensive potencies is difficult since the slopes of the dose-response relationships to clonidine and FLA-136 diverge with increasing dose. Centrally administered FLA-136 caused a rapid fall in blood pressure suggesting that the compound *per se* possesses hypotensive activity and does not, as suggested by Timmermans *et al.* (1979), require the formation of active metabolite(s). By the intravenous route FLA-136 is about 30 times less active and the maximum hypotensive effect is obtained more slowly than after central administration, whilst clonidine is approximately equiactive as a hypotensive agent by both routes. These results may reflect the ease with which clonidine, but not FLA-136, penetrates the blood-brain barrier.

Following oral and intravenous administration, FLA-136 differes from clonidine by lack of activity at both vascular postsynaptic α-adrenoceptors and presynaptic α_2-adrenoceptors located on the nerve endings of the noradrenergic innervation to the blood

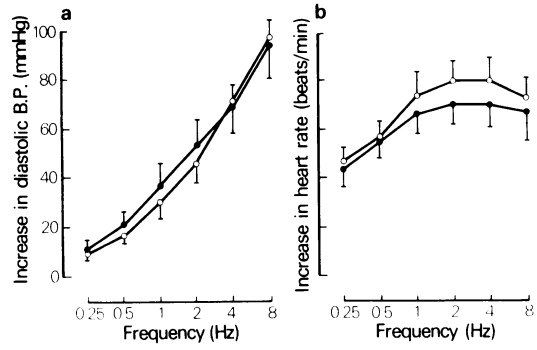

Figure 6 Increases in (a) diastolic blood pressure (mmHg) and (b) heart rate (beats/min) to stimulation of the spinal sympathetic outflow at various frequencies (Hz) in 5 pithed rats before (○) and after (●) FLA-136 (0.3 mg/kg i.v.). Vertical bars indicate s.e.mean.

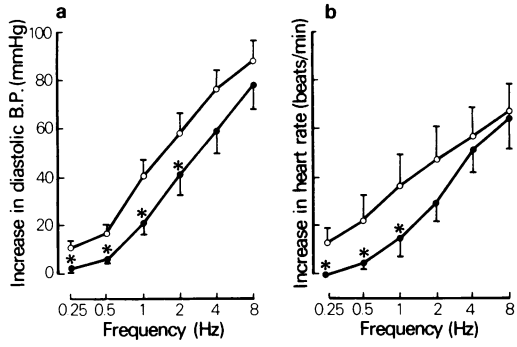

Figure 7 As described for Figure 6 but with clonidine (30 μg/kg i.v.) instead of FLA-136. Significance of difference from control values indicated by: $*P < 0.05$.

vessels and the heart. Our evidence therefore suggests that neither FLA-136 *per se* nor its metabolites have any agonist activity at peripheral presynaptic receptors contrasting with the findings of Timmermans *et al.* (1979) who implicated metabolite(s) of FLA-136 as mediators of its inhibitory activity at cardiac nerve endings in the rat.

Some notable differences have been discovered between the mechanism of hypotensive action of centrally administered FLA-136 and clonidine in the anaesthetized rat. Yohimbine (an α-adrenoceptor blocking drug with some selectivity for peripheral presynaptic α_2-adrenoceptors) antagonized the cardiovascular effects of both FLA-136 and clonidine whereas prazosin (a selective postsynaptic α_1-adrenoceptor antagonist) reduced these responses of clonidine but not of FLA-136. This suggests that central α-adrenoceptors having similar characteristics to peripheral presynaptic α_2-adrenoceptors (yohimbine- and piperoxan-sensitive) are involved in the mediation of the cardiovascular effects of centrally administered FLA-136; Timmermans *et al.* (1979) drew similar conclusions from their studies using intravenously administered FLA-136 in the rat. The central prazosin-sensitive α-adrenoceptors also involved in the mediation of clonidine-induced hypotension and bradycardia are probably, as suggested by Cavero & Roach (1978), similar to vascular postsynaptic α_1-adrenoceptors. In another situation Anden *et al.* (1977) have shown that in spinal rats, clonidine, but not FLA-136, stimulated postsynaptic α-adrenoceptors in the central nervous system as shown by its ability to increase flexor reflex activity.

In rats pretreated centrally with 6-OHDA to destroy noradrenergic neurones, the cardiovascular effects of centrally administered FLA-136 were markedly reduced indicating that the α-adrenoceptors responsible are located presynaptically (α-autoreceptors). This finding is in accord with the biochemical studies of Anden *et al.* (1977) that FLA-136, like clonidine (Anden, Grabowska & Strömbom, 1976), decelerates noradrenaline turnover in rat brain by stimulation of yohimbine-sensitive α-adrenoceptors. The residual hypotensive response to FLA-136 in rats treated with 6-OHDA may reflect the existence of some yohimbine-sensitive α-adrenoceptors postsynaptically or incomplete destruction of noradrenergic neurones by 6-OHDA. A slight reduction in the cardiovascular effects of i.c.v. clonidine following this pretreatment is similar to the findings of Finch, Buckingham, Moore & Bucher (1975) in DOCA/saline hypertensive rats, indicating that in the rat the central α-adrenoceptors (sensitive to both yohimbine and prazosin) mediating the cardiovascular effects of clonidine are largely located postsynaptically. Evidence from binding studies also supports the location of 2 types of α-adrenoceptors postsynaptically in the brain (U'Prichard, Charness, Robertson & Snyder, 1978).

Reduction in the cardiovascular effects of centrally administered clonidine, but not FLA-136, by mianserin (100 μg i.c.v.), suggests that at this dose mianserin is acting as an antagonist at central postsynaptic α-adrenoceptors but lacks blocking activity at central α-autoreceptors. It seems unlikely that the effect of mianserin on clonidine responses is due to its anti-histaminic or anti-5-hydroxytryptamine (5-HT) properties (Brogden, Heel, Speight & Avery, 1978) since metiamide (antagonist of histamine [H_2]-receptors) antagonized the cardiovascular effects of both clonidine and FLA-136 and another 5-HT antagonist, methysergide, failed to affect the response to orally administered clonidine in SH-rats (Robson, Antonaccio, Saelens & Liebman, 1978).

Since the opiate antagonist, naloxone, inhibits the cardiovascular responses evoked by centrally administered clonidine, but not FLA-136, opiate receptors may have a direct or indirect role in the mediation of the response to clonidine in the rat; Farsang & Kunos (1979) drew similar conclusions from their studies in conscious SH-rats. However, this interaction may be limited to the rat since in the dog (Daskalopoulos, Schmitt & Laubie, 1975) and man (Brown, Dollery, Fitzgerald, Watkins & Zamboulis, 1980) naloxone did not antagonize the cardiovascular effects of clonidine. Since the cardiovascular effects of clonidine, but not FLA-136, are reduced by the opiate antagonist, stimulation of central postsynaptic α-adrenoceptors, rather than of central α-autoreceptors, may result in the release of endogenous opiate to modulate indirectly the cardiovascular effects of clonidine. This hypothesis is supported by binding studies, using rat brain, which do not provide evidence that such an interaction occurs at a common receptor (Farsang & Kunos, 1979; Golembiowska-Nikitin, Pilc & Vetulani, 1980).

The ability of metiamide to inhibit the hypotension and bradycardia produced by both FLA-136 and clonidine suggests that H_2-receptors may be involved as mediators of the above responses. Others (Karppanan *et al.*, 1976; Finch *et al.* 1978; Borkowski & Finch, 1979) have also shown that i.c.v. administrations of metiamide are effective in reversing the hypotension induced by clonidine in the rat whereas diphenhydramine (H_1-blocker) is ineffective (Karppanen *et al.* 1976). Work in the pithed rat (Doxey & Everitt, 1979), and binding studies with [^{3}H]-clonidine (Pilc, Golembiowska-Nikitin & Vetulani, 1979) and [^{3}H]-prazosin (Timmermans, Karamat Ali, Kossen & Van Zwieten, 1980), suggests that metiamide does not antagonize the cardiovascular effects of clonidine and FLA-136 by blocking α-adrenoceptors but that the effector pathways for

these actions of clonidine and FLA-136 involve a histaminergic component.

Our experiments involving i.c.v. administration of clonidine to conscious rats confirm earlier reports that clonidine causes sedation (Holman, Shillito & Vogt, 1971; Tsoucaris-Kupfer & Schmitt, 1972; Cavero & Roach, 1978; Drew *et al.* 1979). FLA-136 (0.3 mg) also shows some activity in conscious rats assessed for overt sedation, a similar effect being produced by clonidine (10 μg). However, at a higher dose the sedative potential of clonidine increases whereas that of FLA-136 diminishes. This latter result suggests that FLA-136 may have some antagonist properties at high doses and does not allow a comparison of the relative activities of the drugs as sedatives and hypotensives.

In conclusion the present results suggest that, in the rat, FLA-136 is a selective stimulant of central α-autoreceptors whilst clonidine stimulates 2 types of central postsynaptic α-adrenoceptors (one yohimbine- and the other prazosin-sensitive). Since FLA-136, unlike clonidine, lacks any action at peripheral pre- or postsynaptic α-adrenoceptors but when administered centrally its responses are antagonized by yohimbine (which is known to act peripherally as an α_2-adrenoceptor antagonist) this may indicate a difference in the nature of central α-autoreceptors mediating the FLA-136 response and peripherally located α_2-adrenoceptors. It is possible that differences between the mechanisms by which FLA-136 and clonidine lower blood pressure may be attributed to action at different receptors. This hypothesis would agree with previous biochemical studies showing that FLA-136 does not displace [^{3}H]-clonidine bound to rat cerebral cortex membranes (Jarrott, Louis & Summers, 1979). In addition to the involvement of central α-adrenoceptors in the cardiovascular responses to FLA-136 and clonidine, central histamine H_2-receptors may also be implicated in such responses whilst opiate receptors may be involved in mediating the hypotension evoked by the central administration of clonidine but not of FLA-136.

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