Evidence that different regional sympathetic outflows vary in their sensitivity to the sympathoinhibitory actions of putative 5-HT_{1A} and α_2 -adrenoceptor agonists in anaesthetized cats

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1 An investigation was carried out to determine whether the centrally acting hypotensive drugs whose mechanisms of action are due either to activation of 5-HT_{1A} receptors (flesinoxan, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and urapidil — also an α_1 -adrenoceptor antagonist) or to activation of α_2 -adrenoceptors (clonidine and moxonidine) cause differential sympathoinhibition.

2 Cats were anaesthetized with α -chloralose and simultaneous recordings were made of whole cardiac, splanchnic and renal nerve activity, blood pressure and heart rate. Cumulative dose-response (i.v.) curves were constructed in separate experiments for the above hypotensive agents on these parameters.

3 Renal nerve activity was found to be more sensitive to the sympathoinhibitory action of flesinoxan and 8-OH-DPAT when compared with cardiac nerve activity, whereas the reverse was observed for clonidine and moxonidine, cardiac being more sensitive than renal nerve activity. Splanchnic nerve activity was similarly affected by all drugs. Furthermore at the highest dose, all drugs tended to cause complete inhibition in all regional sympathetic nerve outflows.

4 Urapidil differed from all the above hypotensive drugs in that it caused a similar degree of sympathoinhibition in all sympathetic outflows at all doses. It is suggested that this may be due to the ability of urapidil to block central α_1 -adrenoceptors in addition to stimulation of 5-HT_{1A} receptors.

Introduction

There is a large amount of evidence to suggest that 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and flesinoxan activate 5-HT_{1A} receptors to cause their hypotensive action (Fozard *et al.*, 1987; McCall *et al.*, 1987; Ramage & Fozard, 1987; Doods *et al.*, 1988; Wouters *et al.*, 1988; Laubie *et al.*, 1989) and that such an action is entirely within the central nervous system. However, only moderate thoracic preganglionic sympathoinhibition is observed in anaesthetized cats (Ramage & Fozard, 1987; Ramage *et al.*, 1988). One explanation for this lack of correlation between the thoracic sympathoinhibitory action and the hypotensive action of 8-OH-DPAT and flesinoxan is that these drugs could be having a more potent sympathoinhibitory action at a different level or levels of sympathetic outflow. In this respect the sympathoinhibitory action of flesinoxan on renal

outflow, when compared with other sympathetic outflows has been shown to be greater (Ramage *et al.*, 1988). However a weakness of this latter investigation was that the recordings made from different sympathetic outflows were carried out in separate animals and further, that some of the outflows were recorded from preganglionic, while others were recorded from postganglionic nerves. In order to overcome these weaknesses the present experiments were carried out in which the sympathoinhibitory actions of flesinoxan and 8-OH-DPAT were studied on cardiac, splanchnic and renal nerve activity recorded simultaneously in the same animal. Further, the effects of other hypotensive agents, urapidil (which has also been shown to bind to 5-HT_{1A} receptors; Fozard & Mir, 1987; Groß *et al.*, 1987), along with clonidine and the newer α_2 -adrenoceptor agonist moxonidine (Armah, 1987) were also studied in the above experimental model. The latter two

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drugs were chosen because it has been suggested (Schmitt, 1975) that the sympathoinhibitory action of clonidine, and therefore other α_2 -adrenoceptor agonists, may also vary from one regional outflow to another.

Preliminary accounts of these observations have been published (Ramage & Wilkinson, 1988a,b).

Methods

Experiments were performed on 27 male adult cats (2.5–4.2 kg) anaesthetized with a mixture of α -chloralose (70 mg kg^{-1} , i.v.) and pentobarbitone sodium (12 mg per animal). Supplementary doses of α -chloralose (10 – 15 mg kg^{-1}) anaesthetic were given as required. The animals were artificially ventilated using positive pressure after neuromuscular blockade with vecuronium bromide ($200 \text{ } \mu\text{g kg}^{-1}$). Arterial blood gases, pH, body temperature, blood pressure and heart rate were monitored as previously described (Ramage, 1984). Drugs were given by bolus injection into the jugular vein.

Simultaneous recordings were made of sympathetic nerve activity from the inferior cardiac (postganglionic), splanchnic (mixed pre- and postganglionic) and renal (postganglionic) nerves. To record cardiac nerve activity the animal was laid on its right side and the second and third ribs removed retropleurally to expose the inferior cardiac nerve. A pneumothorax was then created between ribs 5 and 6 to reduce mechanical movement of the rib cage. At this stage the respiration pump was connected to an expiratory resistance of $0.5 \text{ cmH}_2\text{O}$ to prevent atelectasis. The splanchnic and renal nerves were exposed by a retroperitoneal approach. Whole nerve activity was recorded by use of bipolar silver hook electrodes from the central cut or crushed ends of these nerves. Nerve activity was quantified by rectifying and integrating the signal above background noise over 5 s with solid state electronic integrators (constructed by Mr A. Charles, Electronic Workshop, RFHSM). The validity of the threshold setting was verified at the end of the experiment after administration of pentobarbitone sodium (60 mg per animal) or by crushing the nerve to block all activity. The output of the integrator was displayed on a Grass polygraph. Sympathetic nerve activity was tested to ensure that it was under baroreceptor modulation, by checking that activity in these nerves was increased by a fall in blood pressure caused by sodium nitroprusside ($2 \text{ } \mu\text{g kg}^{-1}$) and decreased by a rise in blood pressure caused by noradrenaline ($0.25 \text{ } \mu\text{g}$ per animal).

In all experiments all parameters were recorded over a 20 min period before injection of the test solutions. In separate experiments cumulative dose-

response curves were constructed for flesinoxan (3 – $300 \text{ } \mu\text{g kg}^{-1}$), 8-OH-DPAT (0.5 – $128 \text{ } \mu\text{g kg}^{-1}$), clonidine (0.3 – $10 \text{ } \mu\text{g kg}^{-1}$), moxonidine (1 – $100 \text{ } \mu\text{g kg}^{-1}$) and urapidil (0.01 – 3.0 mg kg^{-1}). Intervals between injections times were: for flesinoxan 5, 10, 15 and 15 min, for 8-OH-DPAT 5, 5, 10 and 10 min, for clonidine 5, 10 and 15 min, for moxonidine 5, 7, 10 and 15 min and for urapidil 5, 5, 10, 10 and 10 min. The time between the injections represents the time taken for the change in blood pressure caused by these drugs to stabilize. All drugs except urapidil were dissolved in saline; urapidil was dissolved in 0.04 M lactic acid. All doses except noradrenaline, urapidil and 8-OH-DPAT refer to the salts of the drugs.

Analysis of data

Baseline values were measured just before the first injection. Responses for all parameters were measured just prior to the next injection. The changes that occurred after the final dose of each drug were measured after 15 min for flesinoxan, 10 min for 8-OH-DPAT and urapidil, and finally 30 min for clonidine and moxonidine. Nerve activity was taken as the mean level over 1 min in arbitrary units. All results are expressed as changes from baseline values. Activity over the complete dose range for all the nerves for each drug was analysed with a two way analysis of variance with replicates. Comparison of nerve activity at each dose was carried out using a least significant difference test (Sokal & Rohlf, 1969). Differences were considered significant when $P < 0.05$.

Drugs

The following drugs were purchased from Sigma, α -chloralose, atropine methonitrate and sodium nitroprusside; 8-OH-DPAT HBr was obtained from RBI: Semat Technical Ltd and pentobarbitone sodium from May & Baker Ltd. The following drugs were kindly donated by the companies indicated, flesinoxan HCl (Duphar B.V.), clonidine HCl (Boehringer Ingelheim), moxonidine HCl $\cdot \text{H}_2\text{O}$ (Beiersdorf AG) and urapidil (Byk Gulden Pharmazeutika).

Results

Effect of cumulative doses of flesinoxan (3 – $300 \text{ } \mu\text{g kg}^{-1}$) over 60 min

Cumulative doses of flesinoxan ($n = 5$) caused dose-related falls in mean blood pressure, reaching a maximum of $-63 \pm 6 \text{ mmHg}$ (mean $\pm$ s.e.mean) from a mean baseline value of $126 \pm 7 \text{ mmHg}$, and

Figure 1 Traces comparing the effect of cumulative i.v. injections of flesinoxan (a) and moxonidine (b) in two separate anaesthetized cats on cardiac nerve activity (CNA), splanchnic nerve activity (SNA), renal nerve activity (RNA) and blood pressure (BP).

in heart rate, reaching $-130 \pm 8 \text{ beats min}^{-1}$ from a mean baseline value of $215 \pm 9 \text{ beats min}^{-1}$ at the highest dose. These falls in mean blood pressure and heart rate were associated with a reduction in cardiac, splanchnic and renal nerve activity of $80 \pm 10\%$, $93 \pm 3\%$ and 100% respectively caused by the highest dose. However, the different sympathetic outflows showed varying sensitivity to the sympathoinhibitory action of flesinoxan (Figure 1). Sympathetic nerve activity first began to decline in the renal and splanchnic nerves followed by the cardiac nerve (Figure 2). At doses of 30 and $100 \mu\text{g kg}^{-1}$ sympathoinhibition in the renal nerve was significantly greater than that in the cardiac nerve ($P < 0.05$: least significant difference two way analysis of variance; Figure 2).

Effect of cumulative doses of 8-OH-DPAT (0.5–128 $\mu\text{g kg}^{-1}$) over 40 min

Cumulative doses of 8-OH-DPAT ($n = 6$) caused dose-related falls in mean blood pressure, reaching a maximum of $-76 \pm 5 \text{ mmHg}$ at the highest two

doses (32 and $128 \mu\text{g kg}^{-1}$) from a mean baseline value of $137 \pm 4 \text{ mmHg}$, and a fall in heart rate of $-128 \pm 12 \text{ beats min}^{-1}$ from a mean baseline value of $220 \pm 11 \text{ beats min}^{-1}$. These falls in mean blood pressure and heart rate were associated with a reduction in cardiac, splanchnic and renal nerve activity of $86 \pm 5\%$, $87 \pm 5\%$ and 100% respectively by the highest dose (Figure 2). 8-OH-DPAT also caused a similar pattern of differential sympathoinhibition to that seen with flesinoxan, sympathetic nerve activity first declining in the renal, followed by splanchnic and then the cardiac nerve. At doses of 8 and $32 \mu\text{g kg}^{-1}$, sympathoinhibition in the renal nerve was significantly greater than that in the cardiac nerve while also at the $32 \mu\text{g kg}^{-1}$ dose sympathoinhibition was greater in renal than in splanchnic nerve activity.

Effect of cumulative doses of urapidil (0.01–3 mg kg^{-1}) over 50 min

From the dose of 0.1 mg kg^{-1} upwards urapidil caused decreases ($n = 6$; Figure 3) in a dose-

Figure 2 Anaesthetized cats: the effect of cumulative i.v. doses of flesinoxan (a; $n = 5$) 8-OH-DPAT (b; $n = 6$) clonidine (c; $n = 5$) and moxonidine (d; $n = 5$) on changes (Δ) in cardiac nerve activity ($\blacklozenge$), splanchnic nerve activity ($\bullet$), renal nerve activity ($\blacksquare$). The changes in blood pressure caused by these drugs are also shown: in (e) 8-OH-DPAT (∇) and flesinoxan ($\blacktriangledown$); and in (f) moxonidine ($\triangle$) and clonidine ($\blacktriangle$). The ordinates are the same as in (a)–(d). Each point represents the mean value and the vertical bars show s.e.mean. * $P < 0.05$; ** $P < 0.01$, comparison between cardiac and renal nerve activity least significant difference two analysis of variance. For clarity, comparison between splanchnic and the other nerves has not been included in the graph; see text for details.

dependant manner in all the parameters being recorded. At the highest dose, mean blood pressure had decreased by 47 ± 4 mmHg from a baseline value of 120 ± 7 mmHg, heart rate by

60 ± 8 beats min^{-1} from a baseline value of 198 ± 10 beats min^{-1} and cardiac, splanchnic and renal nerve activity by $89 \pm 5\%$, $93 \pm 3\%$ and $95 \pm 1\%$ respectively. However, the different sympa-

Figure 3 Anaesthetized cats: the effect of cumulative i.v. doses of urapidil ($n = 6$) on changes (Δ) in cardiac nerve activity ($\diamond$), splanchnic nerve activity ($\circ$), renal nerve activity ($\square$), heart rate (HR) and blood pressure (BP). Each point represents the mean value and the vertical bars show s.e.mean.

thetic outflows did not show any varying sensitivity to the sympathoinhibitory action of urapidil. An injection of atropine methonitrate (0.1 mg kg^{-1}) at the end of the experiments caused a rise in heart rate of $26 \pm 4 \text{ beats min}^{-1}$.

Effect of cumulative doses of clonidine (0.3 – $10 \mu\text{g kg}^{-1}$) over 60 min

Cumulative doses of clonidine ($n = 5$) caused dose-related falls in mean blood pressure, reaching a maximum of $-56 \pm 8 \text{ mmHg}$ from a mean baseline value of $149 \pm 12 \text{ mmHg}$ and in heart rate, reaching $-68 \pm 11 \text{ beats min}^{-1}$ from a mean baseline value of $234 \pm 10 \text{ beats min}^{-1}$, at the highest dose. These falls in mean blood pressure and heart rate were associ-

ated with a reduction in cardiac, splanchnic and renal nerve activity of $97 \pm 2\%$, $84 \pm 5\%$ and $77 \pm 6\%$ respectively by the highest dose (Figure 2). The different sympathetic outflows did show varying sensitivity to the sympathoinhibitory action of clonidine but this was in the reverse order to that which had been observed for flesinoxan and 8-OH-DPAT. Overall, cardiac nerve activity was the most sensitive while renal nerve activity was the least. At the doses of 1, 3 and $10 \mu\text{g kg}^{-1}$ clonidine caused a significantly greater degree of inhibition of cardiac nerve activity than of renal nerve activity. Also at a dose of $3 \mu\text{g kg}^{-1}$, sympathoinhibition was significantly greater in the cardiac nerve than in the splanchnic nerve. At doses of 0.3 and $1 \mu\text{g kg}^{-1}$, sympathoinhibition was significantly greater in the splanchnic than in the renal nerve.

Effect of cumulative doses of moxonidine (1 – $100 \mu\text{g kg}^{-1}$) over 67 min

Cumulative doses of moxonidine ($n = 5$) caused dose-related falls in mean blood pressure, reaching a maximum of $-62 \pm 3 \text{ mmHg}$, from a mean baseline value of $142 \pm 2 \text{ mmHg}$, and in heart rate, reaching $-132 \pm 12 \text{ beats min}^{-1}$ from a mean baseline value of $260 \pm 3 \text{ beats min}^{-1}$ at the highest dose. These falls in mean blood pressure and heart rate were associated with a 100% reduction in cardiac, splanchnic and renal nerve activity by the highest dose (Figure 1). Moxonidine also caused a similar pattern of differential sympathoinhibition to that seen with clonidine, sympathetic nerve activity declining first in the cardiac, followed by splanchnic and then the renal nerve (Figure 2). Over the dose-range 1 – $30 \mu\text{g kg}^{-1}$ moxonidine had a significantly greater sympathoinhibitory action on cardiac than on renal nerve activity (Figure 1). Moxonidine also had a significantly greater sympathoinhibitory action on splanchnic than on renal nerve activity over the dose-range 1 – $10 \mu\text{g kg}^{-1}$. Only at a dose of $3 \mu\text{g kg}^{-1}$ was the sympathoinhibitory action of moxonidine significantly greater on cardiac than on splanchnic nerve activity.

Discussion

The present results demonstrate that different sympathetic outflows vary in their sensitivity to the sympathoinhibitory actions of flesinoxan, 8-OH-DPAT, clonidine and moxonidine, when administered intravenously to anaesthetized cats. Renal nerve activity was found to be more sensitive to the sympathoinhibitory action of flesinoxan and 8-OH-DPAT than cardiac nerve activity, whereas the reverse was observed for clonidine and moxonidine, cardiac

being more sensitive than renal nerve activity. However, all these four drugs caused a similar depression of splanchnic nerve activity. Urapidil differed from these four drugs in that it caused a uniform reduction in sympathetic nerve activity at all the levels being recorded. These observations combined with the large amount of evidence that descending projections can selectively influence certain sympathetic outflows (Futuro-Neto & Coote, 1982b; Barman *et al.*, 1984; Meckler & Weaver, 1985; Stein *et al.*, 1989) suggest that such patterning may be dependent on the chemical transmitter that is released by a particular descending pathway.

5-HT-containing neurones, which are found in the raphe and the ventrolateral medulla, have been shown to project to preganglionic sympathetic motoneurons in the intermediolateral cell column (Bowker *et al.*, 1981; Loewy & Neil, 1981; Jacobs *et al.*, 1984; Marson & Loewy 1985; Ciriello *et al.*, 1986; 1988). These areas in the cat brain have also been shown to contain 5-HT_{1A} binding sites (Dashwood *et al.*, 1988a,b). As flesinoxan and 8-OH-DPAT are agonists at 5-HT_{1A} receptors (see Introduction) the present results would suggest that the above regions may be more important in the control of renal sympathetic outflow than other sympathetic outflows. In this respect electrical stimulation of the caudal part of the nucleus raphe obscurus can cause reduction or abolition of renal nerve activity with simultaneous facilitation of sympathetic nerve activity to the skeletal muscle vascular bed (Futuro-Neto & Coote, 1982b). The effect of flesinoxan and 8-OH-DPAT on skeletal muscle sympathetic nerve activity is unknown, however, both drugs cause profound hypotension associated with little change in femoral arterial conductance (Ramage & Fozard, 1987; Ramage *et al.*, 1988) suggesting, indirectly, that these drugs do not cause inhibition of skeletal muscle sympathetic nerve activity. Furthermore, Barman *et al.* (1984) showed that 50% of neurones in the medullary raphe and 30% in the ventrolateral medulla showed activity which was differential, related either to renal or cardiac nerve activity. In the rostral ventrolateral medulla, application of glycine was shown to cause a much greater reduction in renal than in mesenteric nerve activity and a small fall in heart rate (Stein *et al.*, 1989). In this respect, in the anaesthetized dog, microinjection of 8-OH-DPAT into the ventrolateral medulla caused a large fall in blood pressure and renal nerve activity but only a small fall in heart rate (Laubie *et al.*, 1989). A similar pattern of sympathoinhibition to that observed for 8-OH-DPAT and flesinoxan in the present experiments, has been reported during periods of desynchronized-like sleep in cats (Futuro-Neto & Coote, 1982b) and depletion of 5-HT significantly reduced the inhibition of renal nerve activity

occurring during desynchronized sleep (Coote *et al.*, 1985).

The present results also show that cardiac nerve activity is more sensitive to the sympathoinhibitory action of clonidine and moxonidine, both of which cause their hypotensive action by stimulating α_2 -adrenoceptors (see Schmidt, 1977; Unnerstall *et al.*, 1984; Gillis *et al.*, 1985; Armah, 1987), than the 5-HT_{1A} agonists, flesinoxan and 8-OH-DPAT. With respect to differential control of cardiac nerve activity there are few observations concerning any particular brainstem area. However, the most sensitive site in terms of the hypotension occurring after microinjections of clonidine, has been shown to be the lateral reticular nucleus which is near to the ventral lateral surface of the brainstem (Gillis *et al.*, 1985). Interestingly α_2 -adrenoceptor binding sites have been shown to be in similar areas of the brain stem (Unnerstall *et al.*, 1984; Dashwood *et al.*, 1985) as observed for 5-HT_{1A} binding sites (see above).

Of the five drugs used in this study, urapidil failed to show any differential pattern of sympathoinhibition although it is known to bind to 5-HT_{1A} receptors. However, the present experiments support the suggestion that urapidil does stimulate central 5-HT_{1A} receptors as urapidil caused an increase in vagal drive to the heart, a characteristic feature of 5-HT_{1A} agonists (Ramage & Fozard, 1987). It should be noted that α -chloralose anaesthetized cats have little or no vagal tone (unpublished observations). However, in prazosin pretreated animals a differential sympathoinhibitory action was not observed for the 5-HT_{1A} agonists 8-OH-DPAT (Ramage, 1988). This may explain why urapidil failed to cause a differential pattern of sympathoinhibition as it is also an α_1 -adrenoceptor antagonist (see Ramage, 1986). In this respect it has been shown that α_1 -adrenoceptor antagonists can block 5-HT excitation of raphe neurones (Baraban & Aghajanian, 1980).

The finding that splanchnic nerve activity seems to be affected equally by all the drugs studied may be explained by the observation that activity in this nerve is only slightly influenced by supra-spinal pathways, as transection of the spinal cord at C1 only slightly reduced the activity in this nerve (Qu *et al.*, 1988). However, the differential sensitivity of different sympathetic outflows observed in this study may just reflect the accessibility of various receptor sites within the CNS to these drugs, when given i.v.

In conclusion, the present results demonstrate that the degree of sympathoinhibition caused by centrally acting antihypertensive drugs when given i.v. depends on the dose of the drug, the particular sympathetic nerve being recorded from and whether they act by selectively stimulating 5-HT_{1A} or α_2 -adrenoceptors. From these observations it is sug-

gested that renal nerve activity is under a greater influence from central 5-hydroxytryptaminergic than noradrenergic pathways, while the opposite is true for cardiac nerve activity.

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