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Evidence that activation of central 5-HT_{2B} receptors causes renal sympathoexcitation in anaesthetized rats

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1 The effects of injections i.c.v. of α-methyl-5-(2-thienylmethoxy)-1H-indole-3-ethanamine (BW723C86; 0.02–2 μmol kg^{-1}), a 5-HT_{2B} receptor agonist, on renal sympathetic and phrenic nerve activity, mean arterial blood pressure and heart rate were investigated in α-chloralose anaesthetized rats pretreated with a peripherally acting 5-HT_2 receptor antagonist.

2 BW723C86 i.c.v. caused a dose-related increase in renal nerve activity reaching a maximum of 67±6%, which at the highest dose was associated with a small and maintained fall in mean arterial blood pressure of 7±3 mmHg. These changes were not associated with any significant changes in heart rate or phrenic nerve activity.

3 BW723C86-evoked increases in renal nerve activity and hypotension were attenuated by pretreatment (i.c.v.) with SB204741 (300 nmol kg^{-1}; a 5-HT_{2B} receptor antagonist) but not by the same dose (i.c.v.) of ketanserin (a 5-HT_{2A} receptor antagonist) or RS102221 (a 5-HT_{2C} receptor antagonist). None of these antagonists alone had any effect on the variables being measured.

4 It is concluded that central 5-HT_{2B} receptors may play a selective role in the control of sympathetic supply to the kidney, which could be important in the central mechanisms involved in blood volume regulation.

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Abbreviations: BP, blood pressure; HR, heart rate; i.c.v., intracerebroventricular; Int., integrated; MAP, mean arterial blood pressure; PEG, polyethylene glycol 400; PNA, phrenic nerve activity; RNA, renal nerve activity

Introduction

In conscious and anaesthetized rats, activation of central 5-HT_2 receptors by administering 5-HT or 5-HT_2 receptor agonists i.c.v. causes the release of vasopressin and a rise in arterial blood pressure (Anderson *et al*., 1992; 1996; Pérgola *et al*., 1993; Knowles & Ramage 1999). Recently, it has been shown that this release of vasopressin involves the stimulation of a central angiotensinergic pathway (Saydoff *et al*., 1996; Knowles & Ramage, 1998a) by the activation of the A subtype of the 5-HT_2 receptor (Knowles & Ramage, 1999). Further, the expected 5-HT_2 receptor-mediated sympathoexcitatory response, which is also mediated by activation of central 5-HT_{2A} receptors, was found to be inhibited by this released vasopressin (Knowles & Ramage, 1999). Surprisingly the ability of vasopressin to inhibit the 5-HT_{2A} receptor-mediated sympathoexcitation involves the indirect activation of central 5-HT_{2B} receptors (Knowles & Ramage, 1999). Very low levels of this subtype of the 5-HT_2 receptor have only recently been identified in the brain (Bonhaus *et al*., 1995; Duxon *et al*., 1997a). Therefore, the present experiments were carried out to further investigate the role of central 5-HT_{2B} receptors in cardiovascular regulation using the selective 5-HT_{2B} receptor agonist BW723C86 (see Kennett *et al*., 1996). The effects of BW723C86 administered i.c.v. on renal nerve activity, mean arterial blood pressure, heart rate and phrenic nerve activity were examined in anaesthetized rats pretreated i.c.v. with 10% PEG (antagonist vehicle), ketanserin (a selective 5-HT_{2A} receptor antagonist, see Bonhaus *et al*., 1995; Prins *et al*., 1997), RS-102221 (a selective 5-HT_{2C} receptor antagonist, Bonhaus *et al*., 1997) or SB204741 (a selective 5-HT_{2B} receptor antagonist, Forbes *et al*., 1995). All rats were pretreated with the peripherally acting non-selective 5-HT_2 receptor antagonist BW501C67 (Mawson & Whittington, 1970; Fuller *et al*., 1986) to prevent any peripheral cardiovascular and respiratory effects due to activation of peripheral 5-HT_2 receptors by leakage of BW723C86 out of the brain. A preliminary account of some of these observations has been presented (Knowles & Ramage, 1998b).

Methods

Experiments were performed on male Sprague-Dawley rats (250–350 g). Anaesthesia was induced with isoflurane (2.5% in oxygen) and maintained with α-chloralose (80 mg kg^{-1}, i.v.). Supplementary doses of α-chloralose (10–20 mg kg^{-1}, i.v.) were given as required. Depth of anaesthesia was assessed by the stability of cardiovascular and respiratory variables being recorded. The left carotid artery was cannulated for the measurement of blood pressure and for sampling arterial blood for analysis of pH and blood gases. Blood pressure was measured using a pressure transducer (Gould Statham P23XL) and the heart rate was derived electronically from the blood pressure signal (Gould Biotach Amplifier). The left jugular vein was cannulated for drug administration and a tracheal cannula was implanted. Body temperature was monitored by a rectal probe and maintained at 36–38°C with a homeothermic blanket system (Harvard). The animals were artificially ventilated (rate 50 min^{-1}, stroke volume 8 ml kg^{-1}) with oxygen enriched room air by use of a positive pressure pump (Harvard Rodent Ventilator 683) and neuromuscular blockade

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was produced with decamethonium (3 mg kg^{-1}, i.v.). Blood samples were taken from a T-piece on the carotid arterial cannula and blood gases and pH were monitored with a Corning 238 pH/blood gas analyser. Blood gases were maintained between 90–130 mmHg P_{O_2}, 40–50 mmHg P_{CO_2} and pH 7.3–7.4. Adjustments of the respiratory pump volume were made as necessary to maintain blood gas and pH balance. Once ventilated, the animals were infused (6 ml kg^{-1} h^{-1}) *via* the jugular vein with a solution comprising 10 ml plasma substitute (gelofusine), 10 ml distilled water, 0.04 g glucose, 0.168 g sodium bicarbonate and 10 mg decamethonium. This was to prevent the development of non-respiratory acidosis and to maintain blood volume and neuromuscular blockade.

Cannulation of the lateral cerebral ventricle

The rats were placed in a stereotaxic head holder and a stainless steel guide cannula (22 gauge) was implanted into the right lateral cerebral ventricle. The co-ordinates used from bregma were 4 mm ventral, 1.5 mm lateral and 1 mm posterior. Drug and vehicle solutions were administered through an i.c.v. injection cannula (28 gauge) attached by a length of polythene tubing to a 100 μl syringe (Hamilton). At the end of the experiment, the cannula placement was confirmed by the administration of 5 μl of 2% pontamine sky blue dye.

Recording of phrenic nerve and renal nerve activity

The right phrenic nerve was exposed by deflecting the scapula forwards and dissecting the nerve clear of overlying muscle and connective tissue. The nerve was placed on a bipolar silver hook electrode and crushed distally to the recording site, as described previously (Dreteler *et al*., 1991). Phrenic nerve activity was quantified by integrating the amplitude and frequency of the action potentials in each inspiratory burst or, if continuous, by integrating the amount of activity in a 5 s period, again using a solid state electronic integrator (Royal Free Medical Electronics), the output of which was displayed on a Gould Statham (Model 2007) pen recorder in arbitrary units (see Shepheard *et al*., 1991). The first method of quantifying phrenic nerve activity gives an indication of both the amounts of activity in each inspiratory burst and the frequency of inspiratory bursts. To maintain phrenic nerve activity, a measure of central inspiratory drive, the blood P_{CO_2} values in these animals were maintained at a slightly higher (40–50 mmHg) level than the physiological norm (35–49 mmHg). This usually locked the rate of phrenic nerve firing to the rate of the animals chest movements caused by the respiration pump and changes in phrenic nerve activity were the result of changes in the size of each inspiratory burst. The right kidney was exposed by a retroperitoneal approach and was deflected laterally to reveal the renal artery and nerve. Renal nerve activity was recorded as previously described (Anderson *et al*., 1992). Renal nerve activity was quantified by integrating the signal above background noise over 5 s with a solid state integrator (Royal Free Medical Electronics). The noise levels were verified at the end of the experiment after the administration of pentobarbitone sodium (20 mg per animal).

At the beginning of each experiment the baroreceptor reflex response was tested by observing whether renal nerve activity and heart rate were reduced by a rise in blood pressure caused by noradrenaline (25 ng per animal, i.v.) and were raised by a reduction in blood pressure caused by sodium nitroprusside (0.6 μg per animal, i.v.). Only preparations with an intact baroreceptor reflex were used.

Experimental protocols

The preparation was allowed to stabilize for 30 min before flushing the i.c.v. cannula with saline (5 μl). Ten minutes after this flush injection of saline, 10% PEG (antagonist vehicle) or antagonist was given i.c.v. This was then followed 5 min later by BW501C67 (i.v., 0.1 mg kg^{-1}); and then 5 min later by BW723C86 or saline given i.c.v. and the response followed for at least 20 min. These pre-treatment times were chosen to allow stabilization of any changes in the variables being recorded caused by these pretreatments. In each rat the cardiovascular response of a single dose to BW723C86 was recorded.

Analysis of results

Baseline values were taken 1 min before the addition of drug or vehicle. All results are expressed as changes from baseline values. Nerve activity was measured as the average of the integrated values over 1 min in arbitrary units and is expressed as the percentage change from baseline. Changes in mean

Table 1 Baseline values of mean arterial blood pressure (MAP), heart rate (HR) and phrenic burst rate in anaesthetized and BW501C67 (0.1 mg kg^{-1}) pretreated rats

Experimental pretreatments	*Group test substance*	n	*MAP* (mmHg)	*HR* (beats min^{-1})	*Phrenic* (bursts min^{-1})
Vehicle† (10% PEG)	Vehicle (10% PEG)	10	112±5	345±13	49±4
Vehicle (10% PEG)		3	102±8	322±13	44±7
	BW723C86 (0.02 μmol kg^{-1})		101±7	326±7	44±7
Vehicle (10% PEG)		5	118±1	336±11	44±4
	BW723C86 (0.2 μmol kg^{-1})		118±2	344±8	44±5
Vehicle (10% PEG)		5	107±2	364±21	40±4
	BW723C86 (2 μmol kg^{-1})		111±3	341±18	42±3
Ketanserin (300 nmol kg^{-1})		5	103±4	368±12	51±1
	BW723C86 (0.2 μmol kg^{-1})		104±6	374±12	45±5
RS102221 (300 nmol kg^{-1})		5	101±3	330±17	51±1
	BW723C86 (0.2 μmol kg^{-1})		105±5	338±17	52±0
SB204741 (300 nmol kg^{-1})		5	103±4	335±7	37±3
	BW723C86 (0.2 μmol kg^{-1})		105±6	337±9	35±4
SB204741 (300 nmol kg^{-1})		5	100±5	348±19	45±4
	BW723C86 (2 μmol kg^{-1})		102±7	341±20	46±5

All drugs and solutions were given i.c.v. †Data previously published (Knowles & Ramage, 1999).

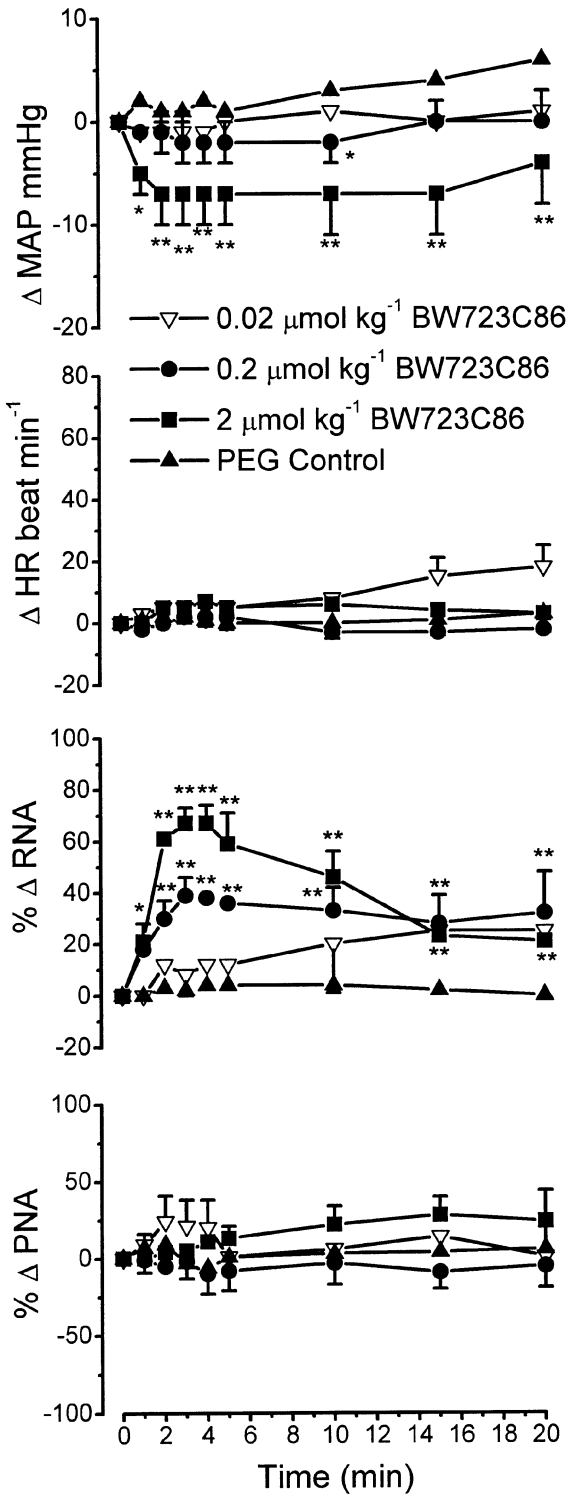

Figure 1 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.): a comparison of the changes from baseline over time (min) caused by three doses of BW723C86 (0.02, 0.2 and 2 μmol kg^{-1}, i.c.v.) in the presence of 10% PEG (5 μl, i.c.v., $n=3$; five and five respectively), in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean. For the lowest dose of BW723C86 the s.e.mean has been removed at the time points 15 and 20 min for RNA for the sake of clarity. Changes caused by BW723C86 (*) were compared with PEG control ($n=10$; data previously published in Knowles & Ramage, 1999, however for the sake of clarity the vertical bars showing s.e.mean have been removed from the points; these are shown in Figure 4) using two-way analysis of variance followed by the least significant difference test to compare the means. *$P<0.05$ and **$P<0.01$.

arterial blood pressure, heart rate, renal and phrenic nerve activity caused by the test drug were compared with time-matched vehicle controls using two-way analysis of variance and the least significant difference test (Sokal & Rohlf, 1969). Changes in variables caused by the antagonist or vehicle pretreatments were compared to the pre-dose baseline using Student's *t*-test for paired data. All values are expressed as the mean$\pm$s.e.mean, differences between means were taken as significant when $P<0.05$.

Drugs and solutions

Drugs were obtained from the following sources: α-chloralose, and decamethonium bromide from Sigma Aldrich Chemical Co., Poole, Dorset, U.K.; noradrenaline acid tartrate from Winthrop, Guildford, Surrey, U.K.; sodium nitroprusside from Sigma Aldrich Chemical Co., Poole, Dorset, U.K.; isoflurane from Abbott Labs. Ltd, Queenborough, Kent, U.K.; Gelofusine from Braun Medical Ltd, Aylesbury, Bucks, U.K.; polyethylene glycol 400 (PEG) from Merck/BDH, Poole, Dorset, U.K.; ketanserin and 8-[5-(5-amino 2,4-dimethoxyphenyl) 5-oxopentyl]-1,3,8-triazaspiro[4,5] decane-2,4-dione (RS 102221) from Research Biochemicals Inc., Semat Technical Ltd, St. Albans, U.K.; α-methyl-5-(2-thienylmethoxy)-1H-indole-3-ethanamine (BW723C86) from Tocris Cookson Ltd, Bristol, U.K.; pentobarbitone sodium from Rhône Mérieux Ltd, Harlow, Essex, U.K. The following were gifts from the sources indicated: α-anilino-N-2-m-chlorophenoxypropylacetamide (BW501C67) from Wellcome Research Laboratories, Beckenham, Kent, U.K., N-(1-methyl-5-indolyl)-*N*′-(3-methyl-5-isothiazolyl) urea (SB204741) from SmithKline Beecham Pharmaceuticals, Harlow, Essex, U.K. Drugs given i.c.v. were dissolved in 0.9% w v^{-1} saline except ketanserin, RS 102221 and SB204741 which were dissolved in 10% PEG. Solutions were administered in a volume of 5 μl over a 20 s period. All drugs given i.v. were dissolved in saline.

Results

Administration of BW501C67 (0.1 mg kg^{-1} $n=33$) i.v. had no effect on baseline variables. In animals pretreated with 5 μl of 10% PEG i.c.v. (PEG control; $n=10$), injection of saline i.c.v. 10 min later also had little effect on mean arterial blood pressure, heart rate, renal or phrenic nerve activity and these variables remained stable for the duration of the experiment (Figures 1 and 4; data previously published, Knowles & Ramage, 1999). However, from the 10 min point onwards mean arterial blood pressure tended to increase reaching 6$\pm$1 mmHg by the end of the experiment. Baseline values for all groups are shown in Table 1.

Effect of i.c.v. administration of BW723C86

In rats pretreated with 10% PEG (i.c.v., $n=3$), BW723C86 (0.02 μmol kg^{-1}, i.c.v.) had no significant effect on mean arterial blood pressure, heart rate, renal nerve activity or phrenic nerve activity, all of which remained stable for the duration of the experiment (Figure 1). However, 0.2 μmol kg^{-1} of BW723C86 (i.c.v., $n=5$) evoked a significant ($P<0.05$) increase in renal nerve activity, when compared to PEG control, after 2 min of 30$\pm$7% reaching a maximum of 39$\pm$7% after 3 min, above baseline values, which was maintained for the duration of the experiment. This renal sympathoexcitation was not associated with any significant changes in mean arterial blood pressure, heart

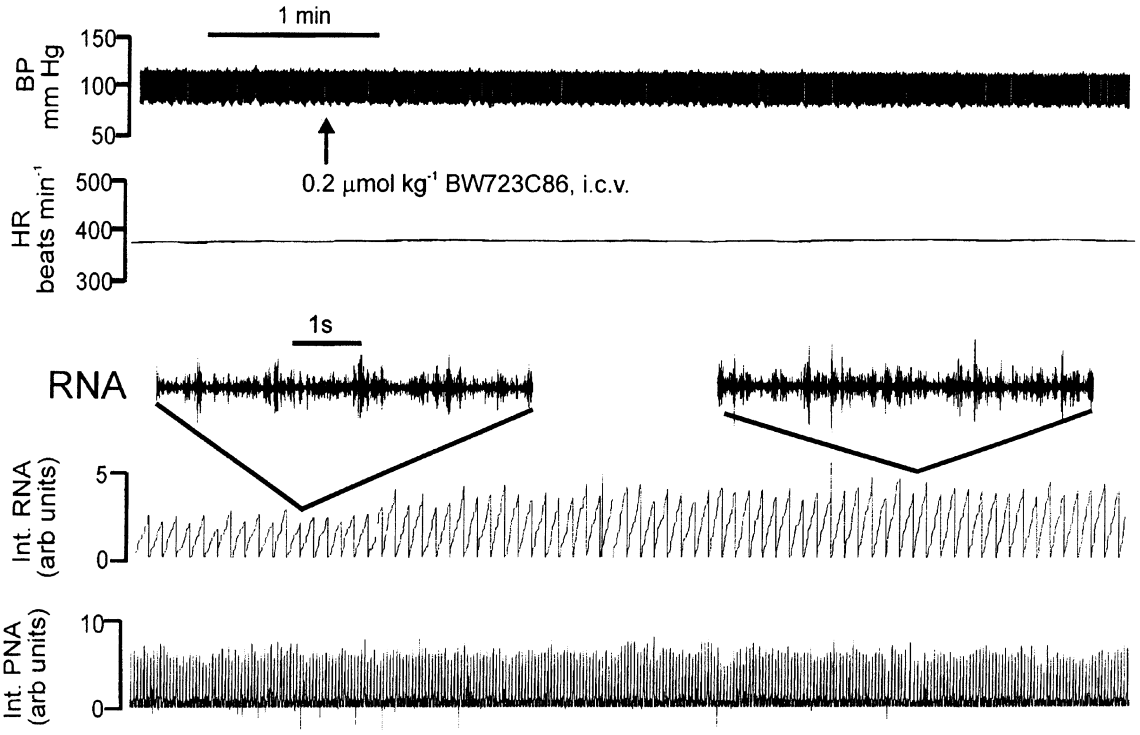

Figure 2 Traces showing the effects of BW723C86 i.c.v.; 0.2 μmol kg^{-1} on arterial blood pressure (BP), heart rate (HR), integrated renal nerve activity (Int. RNA) plus the recorded renal nerve activity for two integrated time points and integrated phrenic nerve activity (Int. PNA) in an anaesthetized neuromuscular blocked and artificially ventilated rat pretreated with i.c.v. 10% PEG. and i.v. BW501C67.

rate or phrenic nerve activity over the first 5 min. Only at the 10 min time point was the change in mean arterial blood pressure of 2 ± 2 mmHg significantly smaller than that observed in the PEG control (see Figure 1). Representative traces from one of these experiments are shown in Figure 2. At the higher dose of 2 μmol kg^{-1} (i.c.v.; $n=5$) BW723C86 again caused a significant renal sympathoexcitation which was maintained. This sympathoexcitation was earlier in onset and significantly larger when compared with 0.2 μmol kg^{-1} BW723C86, reaching a maximum of $67\pm6\%$, also after 2 min. Although there were no associated changes in heart rate and phrenic nerve activity, mean arterial blood pressure did significantly decline after 1 min reaching a maximum by 2 min of -7 ± 3 mmHg, which was maintained over the duration of the experiment (see Figure 1).

In three experiments, 2 μmol kg^{-1} BW723C86 was administered i.v. in anaesthetized rats which had been pretreated with the peripheral 5-HT$_2$ receptor antagonist BW501C67 and was found not to have any effect on the above variables.

Effect of i.c.v. administration of BW723C86 on baseline variables in rats pretreated (i.c.v.) with Ketanserin, RS102221 or SB 204741

None of the antagonists had any effect on baseline variables (see Table 1).

Pretreatment with ketanserin (300 nmol kg^{-1}, i.c.v., n=5)

BW723C86 (0.2 μmol kg^{-1}, i.c.v., $n=5$) in the presence of ketanserin, still evoked a similarly maintained renal sympathoexcitation when compared to BW723C86 alone ($35\pm6\%$ c.f. $39\pm7\%$, Figure 3). Again BW723C86 had no significant effect on any of the other variables being recorded during the first 5 min. However, the small change in mean arterial blood pressure was not observed at the 10 min time point and there was now a small and significant, sustained increase in heart rate of 10 ± 4 beats min^{-1} when compared to BW723C86 alone but not when compared to PEG control.

Pretreatment with RS 102221 (300 nmol kg^{-1}, i.c.v., n=5).

In the presence of RS 102221, i.c.v. injection of BW723C86 (0.2 μmol kg^{-1}, $n=5$) still evoked a similar maintained increase in renal nerve activity ($37\pm2\%$ *c.f.* $39\pm7\%$ after 3 min see Figure 3). However, BW723C86 now caused a small and sustained fall in mean arterial blood pressure from the 3 min time point of -2 ± 2 mmHg when compared to PEG control and after 10 min this fall was associated with a small and significant tachycardia of 18 ± 4 beats min^{-1}. This tachycardia was then maintained over the remaining duration of the experiment. Phrenic burst size was also significantly increased at the 15 and 20 min time points when compared with BW723C86 in the presence of PEG.

Pretreatment with SB 204741 (300 nmol kg^{-1}, i.c.v., n=5)

SB 204741 significantly attenuated the renal sympathoexcitation evoked by 0.2 μmol kg^{-1} of BW723C86 (i.c.v., see Figure 3) compared to BW723C86 alone. This was also true for the higher dose of 2 μmol kg^{-1} of BW723C86 (i.c.v; $n=5$) where pretreatment with SB 204741 also attenuated the renal sympathoexcitation as well as the fall in mean blood pressure (Figure 4). However, the high dose of BW723C86 still caused a small sympathoexcitation and fall in mean arterial pressure,

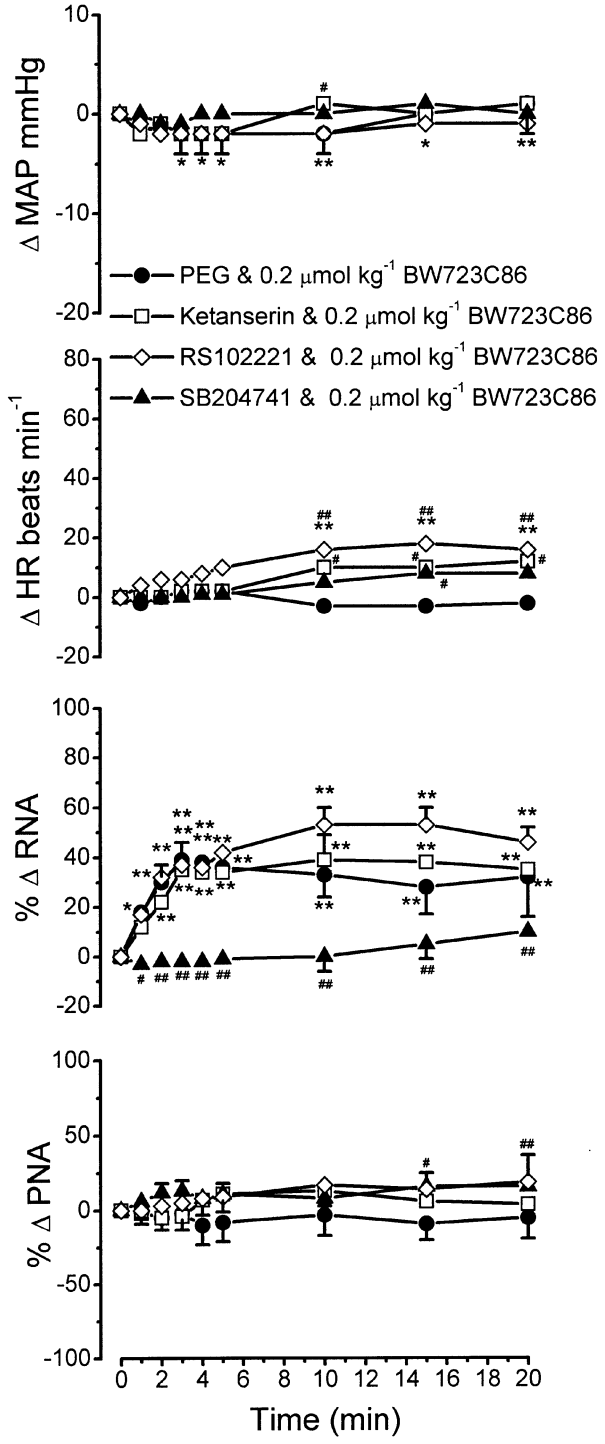

Figure 3 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.): a comparison of the changes from baseline over time (min) caused by BW723C86 (0.2 μmol kg^{-1}, i.c.v.) in the presence of ketanserin (300 nmol kg^{-1}, i.c.v., $n=5$), RS102221 (300 nmol kg^{-1}, i.c.v., $n=5$), or SB204741 (300 nmol kg^{-1}, i.c.v., $n=5$) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean. It should be noted that the data for changes caused by BW723C86 in the presence of PEG on mean arterial blood pressure have been obscured by the data points from the other pretreatments. Changes caused by BW723C86 pretreated with antagonists are compared to PEG control ($n=10$*; data not shown for the sake of clarity, see Figure 4) and to BW723C86 (#) using two-way analysis of variance followed by the least significant difference test to compare the means. #, *$P<0.05$ and ##, **$P<0.01$.

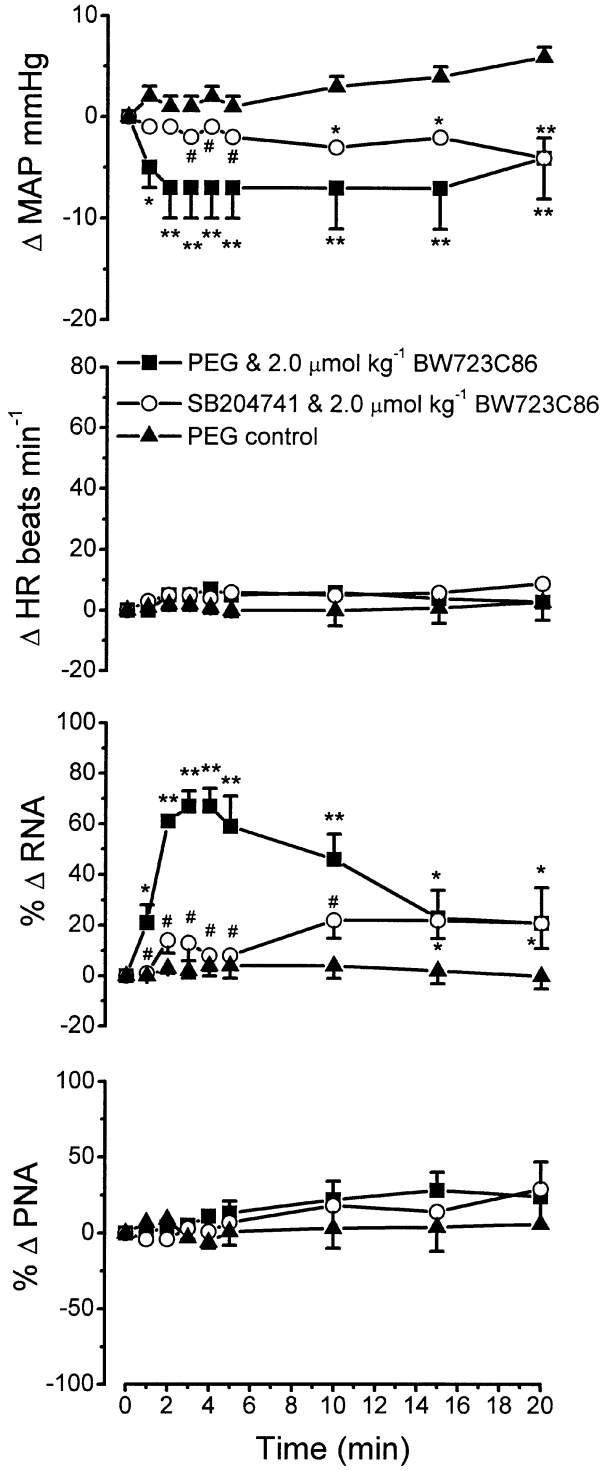

Figure 4 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.): a comparison of the changes from baseline over time (min) caused by BW723C86 (2 μmol kg^{-1}, i.c.v.), BW723C86 (2 μmol kg^{-1}, i.c.v.) in the presence of SB204741 (300 nmol kg^{-1}, i.c.v., $n=5$) and PEG control (5 μl, i.c.v., $n=10$) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean. Changes caused by BW723C86 are compared with PEG control (*, data previously published in Knowles & Ramage, 1999) and with BW723C86 pretreated with SB204741 (#) using two-way analysis of variance followed by the least significant difference test to compare the means. #, *$P<0.05$ and ##, **$P<0.01$.

which were significant when compared with PEG control (Figure 4). In the presence of SB 204741, neither dose of BW723C86 had any effect on heart rate or phrenic nerve activity.

Discussion

BW723C86 a selective 5-HT$_{2B}$ receptor agonist (see Kennett *et al*., 1996) injected into the third ventricle of neuromuscular blocked anaesthetized rats caused a dose-related increase in renal nerve activity. This was associated with no change in heart rate or phrenic nerve activity. However, at the high dose, there was a maintained and significant small fall in mean arterial blood pressure. It is possible that part of the renal nerve sympathoexcitation, at least for the high dose, could be baroreceptor mediated in response to the fall in arterial blood pressure. However, for the medium dose the renal sympathoexcitation was not, at least for the first 5 min, associated with a fall in arterial blood pressure, and further, if a baroreceptor reflex had been initiated, heart rate would also have been expected to increase and this was not observed. The fact that these experiments were carried out in the presence of a peripheral 5-HT$_2$ receptor antagonist and the failure of the large dose of BW723C86, administered i.v. to affect any of the variables being monitored indicates that the effects of BW723C86 on renal nerve activity and arterial blood pressure are due to a central action. Interestingly, compared to other 5-HT$_2$ receptor agonists BW723C86 failed to affect central respiratory drive (Knowles & Ramage, 1999).

The ability of SB204741, a selective 5-HT$_{2B}$ receptor antagonist (Forbes *et al*., 1995), to attenuate the renal sympathoexcitation and hypotension indicates that these effects are mediated by activation of 5-HT$_{2B}$ receptors. The dose of SB204741 used has previously been shown to interfere with central 5-HT$_{2B}$ receptors (Knowles & Ramage, 1999). SB204741 has a very low affinity for 5-HT$_{2A}$ receptors (pK_i of 5; see Forbes *et al*., 1995), nevertheless it does have some affinity for 5-HT$_{2C}$ receptors (pK_i of 5.8 *c.f*. 7.85 at 5-HT$_{2B}$ receptors, Forbes *et al*., 1995). In this respect BW723C86 also only has a 10 fold lower affinity for the 5-HT$_{2C}$ receptor (pK_i of 6.9 *c.f*. 7.9 at 5-HT$_{2B}$ receptors, see Kennett *et al*., 1996). However, the failure of pretreatment with RS102221, a selective 5-HT$_{2C}$ receptor antagonist (Bonhaus *et al*., 1997), to affect renal sympathoexcitation at the same dose as that used for SB204741 supports the above conclusion that this effect of BW723C67 is mediated by activation of 5-HT$_{2B}$ receptors. However, in the presence of RS102221, BW723C86 caused a delayed tachycardia and at this dose (0.2 μmol kg^{-1}) of BW723C86 a small fall in mean arterial blood pressure was now observed within the first 5 min, similar to that seen with the highest dose of BW723C86. In addition there was also a delayed increase in phrenic burst size. These observations suggest that BW723C86 can also activate 5-HT$_{2C}$ receptors. Further, the ability of pretreatment with RS102221 to uncover this hypotension effect for the lower dose of BW723C86 supports the conclusion that the hypotensive response evoked by BW723C86, at the highest dose, is mediated by activation of 5-HT$_{2B}$ receptors. Overall these data suggest that BW723C86 may be also able to activate 5-HT$_{2C}$ receptors at the doses used and further activation of central 5-HT$_{2C}$ receptors *via* the i.c.v. route would be expected to cause a rise in arterial blood pressure. In this respect, in the present experimental system, mCPP (0.2 μmol kg^{-1}) a compound which has been used to activate 5-HT$_{2C}$ receptors in rats (see Curzon & Kennett, 1990) has been reported to cause a small rise in mean arterial blood pressure of 9 ± 2 mmHg (Knowles, 1999).

The failure of the selective 5-HT$_{2A}$ receptor antagonist ketanserin (see Bonhaus *et al*., 1995; Prins *et al*., 1997) to interfere with the BW723C86-mediated sympathoexcitation indicates that the 5-HT$_{2B}$ receptor-mediated sympathoexcitation does not involve the additional activation of 5-HT$_{2A}$ receptors. This is particularly interesting in that activation of 5-HT$_{2A}$ receptors *via* the i.c.v. route causes generalized sympathoexcitation, whereas activation of the 2B subtype would seem to cause only excitation of the renal sympathetic outflow. It could be argued that ketanserin at the dose used may not have blocked 5-HT$_{2A}$ receptors. This is very doubtful as ketanserin has the same affinity as cinanersin for 5-HT$_{2A}$ receptors (see Prins *et al*., 1997) and, at the same dose used for ketanserin, cinanserin under the same experimental conditions has been shown to block central 5-HT$_{2A}$ receptors (Knowles & Ramage, 1999). However, the ability of 5-HT$_{2B}$ receptor activation to cause renal sympathoexcitation is somewhat surprising as these receptors have been previously demonstrated to be involved in the ability of vasopressin to inhibit 5-HT$_{2A}$ receptor-mediated generalized sympathoexcitation (Knowles & Ramage, 1999) suggesting that they have a central sympathoinhibitory action. A possible explanation for this is that there may be at least two different sites in the brain at which activation of 5-HT$_{2B}$ receptors can effect central cardiovascular regulation. One site where 5-HT$_{2B}$ receptor activation causes renal sympathoexcitation and another, at which such activation causes hypotension, presumably by causing sympathoinhibition to resistance vessels. The rapid onset of the renal sympathoexcitation caused by BW723C86 when administered i.c.v. suggests a brain area close to the lateral or third ventricles such as the dorsal hypothalamus and/or medial amygdala in which 5-HT$_{2B}$ receptors have been identified (Duxon *et al*., 1997a). However, which areas are responsible for renal sympathoexcitation and for sympathoinhibition remains to be determined. This sympathoinhibition could be occurring in another sympathetic outflow such as the lumbar or even in the renal nerve itself. In this respect, renal efferent sympathetic nerve activity has other functions than the control of renal vascular resistance, such as renin release and the control of renal tubular absorption (see Dibona & Kopp, 1997). This may explain why the middle dose of BW723C86 failed to increase arterial blood pressure although it did increase renal nerve activity. If this increase in nerve activity had caused an increase in renal vascular resistance and as the kidney receives about 20% of the cardiac output, a rise in arterial blood pressure would have been expected, but if activity had gone up only in sympathetic nerves controlling renal tubular absorption then no pressure change would have been seen. As whole nerve activity was being measured in the present experiments, it would be impossible to observe any differential change in activity. Thus, experiments measuring end organ kidney function now need to be carried out to investigate this possibility.

In conclusion, this is the first study to demonstrate that BW723C86 has central cardiovascular actions, although previously it has been reported to have an anxiolytic action (Kennett *et al*., 1996; 1998; Duxon *et al*., 1997b) and affect feeding behaviour (Kennett *et al*., 1997). Further, these cardiovascular effects have been demonstrated to be due to activation of mainly 5-HT$_{2B}$ receptors, although a small effect at 5-HT$_{2C}$ receptors seems to be evident. The question arises as to the possible role for the 5-HT$_{2B}$ receptors in cardiovascular regulation especially as they are sparsely distributed in the brain. Activation of 5-HT$_{2B}$ receptors causes sympathoexcitation in renal sympathetic outflow but not in that to the heart or to resistance vessels as indicated by the failure of BW723C86

to increase heart rate and mean arterial blood pressure. Further, previous data indicate that these receptors are also involved in the effects of circulating vasopressin on the brain (Knowles & Ramage, 1999). It is therefore suggested that these receptors may be playing a role in the mechanisms by which the brain regulates blood volume.

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