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Evidence for a role for central 5-HT_{2B} as well as 5-HT_{2A} receptors in cardiovascular regulation in anaesthetized rats

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1 The effects of injections i.c.v. of quipazine, (2 μmol kg^{-1}) and 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI; 2 μmol kg^{-1}) on renal sympathetic and phrenic nerve activity, mean arterial blood pressure (MAP) and heart rate were investigated in α-chloralose anaesthetized rats pretreated with a peripherally acting 5-HT_2 receptor antagonist.

2 Quipazine or DOI caused a rise in MAP which was associated with a tachycardia and renal sympathoinhibition in rats pretreated (i.c.v.) with the antagonist vehicle 10% PEG. These effects of quipazine were completely blocked by pretreatment with cinanserin (a 5-HT_2 receptor antagonist) and attenuated by spiperone (a 5-HT_{2A} receptor antagonist). However, pretreatment with SB200646A (a 5-$HT_{2B/2C}$ receptor antagonist) only blocked the sympathoinhibition, while pretreatment with SB204741 (a 5-HT_{2B} receptor antagonist) reversed the sympathoinhibition to excitation as it also did for DOI. Quipazine also caused renal sympathoexcitation in the presence (i.v.) of a vasopressin V_1 receptor antagonist.

3 Injection (i.v.) of the V_1 receptor antagonist at the peak pressor response evoked by quipazine alone and in the presence of SB204741 caused an immediate fall in MAP. For quipazine alone the renal sympathoinhibition was slowly reversed to an excitation, while the renal sympathoexcitation observed in the presence of SB204741 was potentiated. In both, the quipazine-evoked tachycardia was unaffected.

4 The data indicate that cardiovascular responses caused by i.c.v. quipazine and DOI are primarily due to activation of central 5-HT_{2A} receptors, which causes the release of vasopressin and a tachycardia. This released vasopressin appears to suppress a 5-HT_{2A} receptor-evoked central increase in sympathetic outflow, which involves the activation of central 5-HT_{2B} receptors indirectly by the released vasopressin.

Keywords: 5-HT_{2B} receptors; 5-HT_{2A} receptors; blood pressure; sympathetic nerve activity; vasopressin V_1-receptors; quipazine; DOI; SB204741; SB200646A; spiperone

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

Introduction

Administration of 5-hydroxytryptamine intracerebroventricularly (i.c.v.) causes a rise in arterial blood pressure, an initial bradycardia and renal sympathoinhibition followed by a tachycardia and renal sympathoexcitation in anaesthetized rats (Anderson *et al*., 1992). This rise in blood pressure, at least initially, is mediated by the release of vasopressin through activation of central 5-HT_2 receptors (Anderson *et al*., 1992; Pérgola *et al*., 1993). The delayed sympathoexcitation and tachycardia are mediated by activation of central 5-HT_{1A} receptors (Anderson *et al*., 1992). However, it is surprising that this sympathoexcitation is not, at least in part, mediated by central 5-HT_2 receptors, as this is considered to be one of the major actions of this 5-HT receptor subtype (see McCall & Clement, 1994). In this respect, administration of the selective 5-HT_2 receptor agonists, quipazine (Zink *et al*., 1990) or DOI (Dedeoğlu & Fisher, 1991) i.c.v. had little effect on heart rate, although they did induce the expected vasopressin-mediated rise in arterial blood pressure. In fact this rise in arterial blood pressure would be expected to cause a baroreceptor reflex-mediated bradycardia. It could, therefore, be inferred from these latter observations that both compounds are causing a degree of sympathoexcitation to counter the baroreflex-mediated sympathoinhibition. However, the possibility that these 5-HT_2 receptor agonists are leaking out of the brain and causing smooth muscle contraction, especially bronchoconstriction and therefore hypoxia, may complicate this interpretation (see Ramage *et al*., 1993). Therefore the present experiments were carried out to further investigate the effects of i.c.v. quipazine and DOI on sympathetic nerve activity in rats pretreated with the peripherally acting 5-HT_2 receptor antagonist BW501C67 (Mawson & Whittington, 1970; Fuller *et al*., 1986). Experiments were also carried out using selective antagonists for 5-HT_{2A}, spiperone (see Bonhaus *et al*., 1995), for 5-$HT_{2B/2C}$, SB200646A (Kennett *et al*., 1994) and for 5-HT_{2B}, SB204741 (Forbes *et al*., 1995) receptors to determine which 5-HT_2 receptor subtypes may be involved in any of the effects observed. A preliminary account of some of these observations has been presented (Knowles *et al*., 1997).

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},

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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 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 Po_2, 40–50 mmHg Pco_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 (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 Pco_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 (initial saline), 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 quipazine, DOI or saline given i.c.v. and the response followed for at least 20 min. The dose of quipazine was chosen from preliminary experiments in non-neuromuscular blocked rats in which the doses of 0.2, 0.6 and 2.0 μmol kg^{-1} were given i.c.v. Only the latter two doses caused a rise in arterial blood pressure of between 12 and 20 mmHg. The highest dose was chosen as it evoked large consistent rises in arterial blood pressure. In animals pretreated with the V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP or atropine methonitrate these drugs were administered i.v. 5 min after the saline flush or 15 min before the administration of quipazine i.c.v. The control carried out for these experiments was an injection of a similar volume of saline instead of atropine or the V_1 receptor antagonist (atropine/V_1 control) i.v. followed 5 min later by PEG and then quipazine. In another series of experiments the V_1 receptor antagonist was administered i.v. 3 min after i.c.v. administration of quipazine. These pre-treatment times were chosen to allow stabilization of any changes in the variables being recorded caused by the administration of these substances. In each rat the cardiovascular response of a single dose of quipazine, DOI or saline 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 was expressed as the percentage change from baseline. Changes in mean 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 were subsequently analysed using the least significant difference test (Sokal & Rohlf, 1969). Changes in variables caused by the antagonist or vehicle pre-treatments were compared to the pre-dose baseline using Student's *t*-test for paired data. All values are expressed as the mean ± 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; [β-Mercapto-β,β-Cyclopentamethylenepropionyl[1], O-Me-Tyr[2], Arg[8]]-Vasopressin, (d(CH_2)$_5$Tyr(Me)AVP) and decamethonium bromide from Sigma Aldrich Chemical Co. (Poole, Dorset, U.K.); noradrenaline acid tartrate from Winthrop (Guildford, Surrey, U.K.); sodium nitroprusside and atropine methonitrate 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.); 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) and cinanserin from Research Biochemicals Inc., Semat Technical Ltd (St. Albans, 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-pyridyl)urea HCl (SB200646A) and N-(1-methyl-5-indolyl)-*N*′-(3-methyl-5-isothiazoyl) urea (SB204741) from SmithKline Beecham Pharmaceuticals (Harlow, Essex, U.K.); spiperone from Janssen Pharmaceuticals (Beerse, Belgium). Drugs given i.c.v. were dissolved in 0.9% w v^{-1} saline except SB200646A, SB204741, cinanserin and spiperone 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}) i.v. ($n=95$) had no effect on baseline variables.

Effect of i.c.v. administration of saline on baseline variables in saline or PEG (i.c.v.) and BW501C67 (i.v.) pretreated animals

In saline (i.c.v.) pretreated animals, saline injected i.c.v. (5 μl; $n=5$; saline control) 10 min later had little effect on mean arterial pressure (MAP), heart rate or renal nerve activity and these variables remained stable for the duration of the experiment (see Figure 1). In animals pretreated with 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 pressure, heart rate, renal or phrenic nerve activity and these variables remained stable for the duration of the experiment (Figure 6). The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before saline i.c.v. pretreatment were 112±5 mmHg, 345±13 beats min^{-1} and 49±4 bursts min^{-1}, while for PEG i.c.v. pretreatment they were 109±4 mmHg, 330±7 beats min^{-1} and 47±5 bursts min^{-1}, respectively.

Effect of i.c.v. administration of Quipazine and DOI

Quipazine ($n=5$) 2 μmol kg^{-1} caused a significant ($P<0.05$) rise in mean arterial pressure after 2 min reaching a maximum of 20±7 mmHg by 10 min returning to near baseline values by 20 min. This was paralleled by significant inhibition of renal nerve activity which reached a maximum, after 3 min, of −65±13% (Figure 1) and remained significantly inhibited for the duration of the experiment. Heart rate and phrenic nerve activity were unaffected over the duration of the experiment. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate were 113±6 mmHg, 372±12 beats min^{-1} and 53±4 bursts min^{-1}, respectively.

In animals pretreated with 10% PEG i.c.v., 2 μmol kg^{-1} of quipazine i.c.v. ($n=8$) caused a significant rise in mean arterial pressure after the first minute reaching a maximum by 2 min of 16±5 mmHg, compared to PEG control, returning to baseline after 15 min. A significant inhibition of renal nerve activity

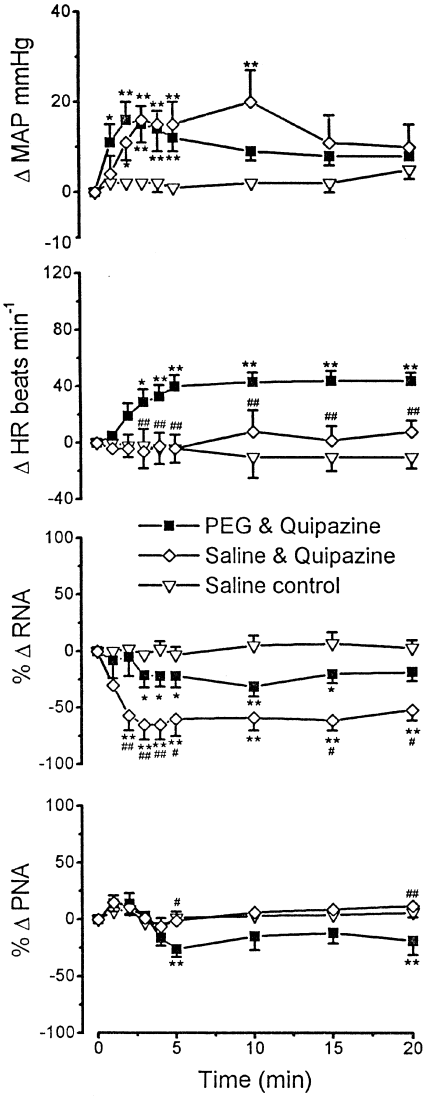

Figure 1 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.): a comparison of the changes from baseline over time (min) caused by quipazine (2 μmol kg^{-1}, i.c.v., in the presence of saline (5 μl, i.c.v., $n=5$), quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of 10% PEG (5 μl, i.c.v., $n=8$) and saline control (5 μl, 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. Changes caused by PEG & Quipazine (*) and Saline & Quipazine (*) were compared with their appropriate controls (PEG control has not been illustrated for the sake of clarity, see Figure 6) and with each other (#) using two-way analysis of variance followed by a least significant difference test to compare the means. *# $P<0.05$ and **## $P<0.01$.

occurred 3 min after injection, reaching a maximum of $-24\pm9\%$ after 10 min (Figure 1). This inhibition was significantly smaller than that observed in rats pretreated with saline. Further, in these PEG pretreated rats, quipazine caused a significant and maintained tachycardia, reaching a maximum of 40 ± 8 beats min^{-1} after 5 min. Changes in phrenic nerve activity were similar to those observed in the PEG control, although there was a significant fall in phrenic nerve activity at 5 min and 20 min of $-26\pm12\%$ and $-19\pm12\%$ respectively. Traces from one of these experiments are shown in Figure 2a. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate were respectively 105 ± 3 mmHg, 304 ± 10 beats min^{-1} and 46 ± 5 bursts min^{-1}.

Injection of DOI (2 μmol kg^{-1}; $n=4$) in PEG pretreated rats caused similar effects to those observed with quipazine, although renal nerve activity declined over the duration of the experiment even though blood pressure had returned to baseline after 15 min. Further, DOI also differed from quipazine in that there was a significant increase in phrenic nerve activity of $35\pm10\%$ and $22\pm4\%$ at 2 and 3 min compared to PEG control (see Figure 4), however when compared to quipazine and PEG, phrenic nerve activity alone, was significantly greater at the 5 and 10 min time points. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate were 120 ± 2 mmHg, 340 ± 10 beats min^{-1} and 48 ± 3 bursts min^{-1}, respectively.

In some of these experiments the same dose of quipazine was injected i.v. at the end of the experiment and found to have no effect on the variables being recorded.

Atropine methonitrate i.v. pretreatment on the i.c.v. quipazine-induced response in animals pretreated with PEG i.c.v.

Atropine methonitrate (0.5 mg kg^{-1}; $n=4$) pretreatment caused a maintained and significant tachycardia of 28 ± 3 beats min^{-1} but did not affect the other variables.

Quipazine (2 μmol kg^{-1}, $n=4$) still caused a significant rise in mean arterial pressure and heart rate reaching maxima of 28 ± 4 mmHg after 3 min and 63 ± 16 beats min^{-1} after 4 min, however renal nerve activity remained unchanged, except for the 15 min time point, compared to atropine control i.e. saline instead of atropine was given i.v. (atropine/V$_1$ control). The rises in mean arterial pressure and heart rate evoked by quipazine were significantly greater than those observed with quipazine alone (atropine/V$_1$ control; $n=4$) at the 3 and 4 min time point (Figure 3). Further, the inhibition of phrenic nerve activity was not attenuated at the 5 and 20 min time points. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection in atropine pretreated rats were respectively 106 ± 5 mmHg, 350 ± 10 beats min^{-1} and 51 ± 5 bursts min^{-1}.

Cinanserin and spiperone pretreatment i.c.v.

Cinanserin i.c.v. (300 nmol kg^{-1}, $n=5$) and spiperone i.c.v (10 and 30 nmol kg^{-1}; $n=5$) failed to have any effect on baseline values.

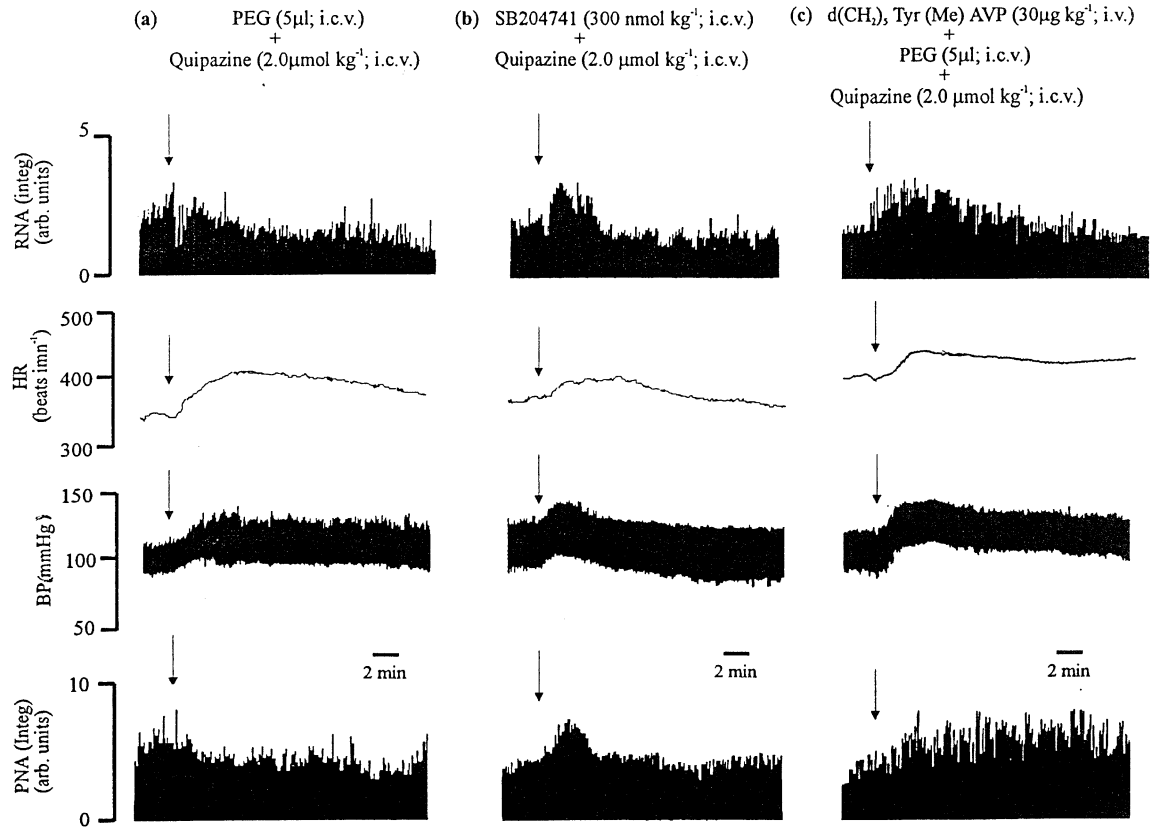

Figure 2 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.). Traces showing the effects of i.c.v. (↓) quipazine (2 μmol kg^{-1}) in the presence of (a) 10% PEG (5 μl, i.c.v.), (b) SB204741 (300 nmol kg^{-1}, i.c.v.) and (c) vasopressin V$_1$ receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP (30 μg kg^{-1}) plus 10% PEG (5 μl, i.c.v.): on arterial blood pressure (BP), heart rate (HR) and integrated (integ.), renal (RNA) and phrenic nerve activity (PNA).

Pretreatment with cinanserin blocked all the effects of i.v.c. quipazine (2 μmol kg^{-1}; $n=5$) on baseline variables, see Figure 4. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection in cinanserin pretreated rats were 109 ± 3 mmHg, 370 ± 16 beats min^{-1} and 43 ± 4 bursts min^{-1} respectively.

The low dose of spiperone attenuated the pressor response and blocked the tachycardia evoked by quipazine (Figure 5). There was also now no associated change in renal and phrenic nerve activity when compared with PEG control. Pretreatment with the high dose of spiperone caused a reversal of the quipazine pressor response to a depressor response reaching a maximum of -9 ± 5 mmHg after 2 min, this fall in pressure was significant when compared with the PEG control. In addition, the tachycardia was significantly attenuated (see Figure 5). The renal sympathoinhibition was also significantly attenuated compared to quipazine alone and there was also no significant change in nerve activity

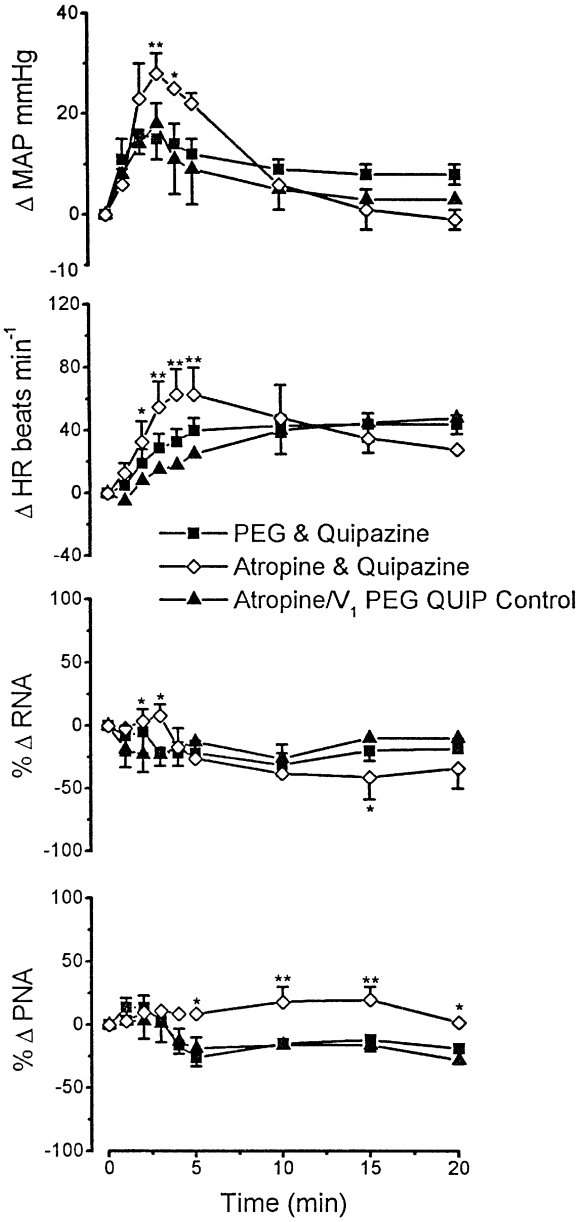

Figure 3 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.): a comparison of the changes from baseline over time (min) caused by quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of 10% PEG (5 μl, i.c.v.) pretreated with atropine methonitrate (0.1 mg kg^{-1}, $n=4$), quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of 10% PEG (5 μl, i.c.v.) pretreated with saline i.v. ($n=4$, atropine/V_1 control) 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 the pretreatment with atropine on PEG & Quipazine responses were compared with the non-pretreated PEG & Quipazine experiments using two-way analysis of variance followed by a least significant difference test to compare the means. *$P<0.05$ and **$P<0.01$.

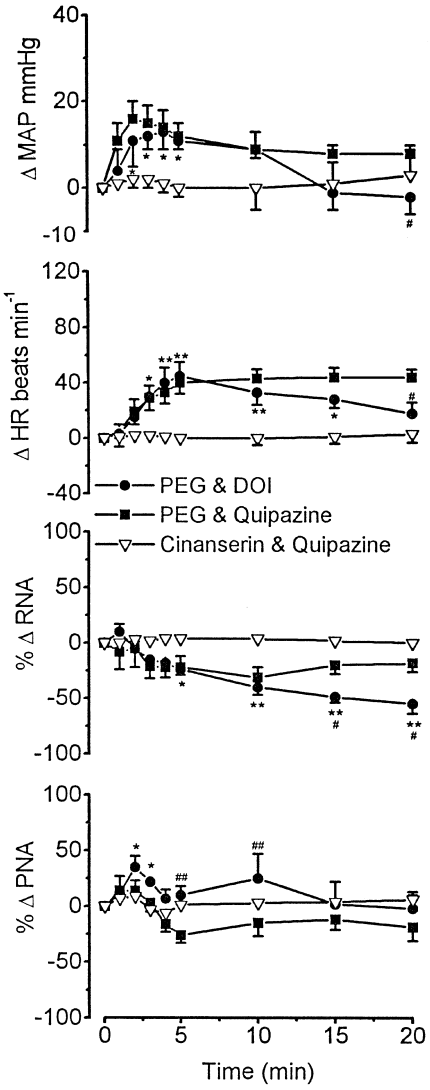

Figure 4 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.): a comparison of the changes from baseline over time (min) caused by DOI (2 μmol kg^{-1}, i.c.v.) in the presence of 10% PEG (5 μl, i.c.v., $n=4$), quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of 10% PEG (5 μl, i.c.v., $n=8$) and quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of cinanserin (300 nmol kg^{-1}, $n=5$), 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. Changes caused by the PEG & DOI were compared with PEG control (*, PEG control data not illustrated for the sake of clarity, see Figure 6) and also PEG & Quipazine (#) while Cinanserin & Quipazine have also been compared with PEG control (no symbol assigned as no data point was significantly different for the comparison indicated) using two-way analysis of variance followed by a least significant difference test to compare the means. *# $P<0.05$ and **## $P<0.01$.

when compared to PEG control. In the presence of the low dose of spiperone, nerve activity tended to increase, becoming significant by the end of experiment. The decrease in phrenic nerve activity at the 5 min time point was blocked. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection in 10 and 30 nmol kg^{-1} spiperone pretreated rats were 110±2 mmHg, 366±15 beats min^{-1} and 46±2 bursts

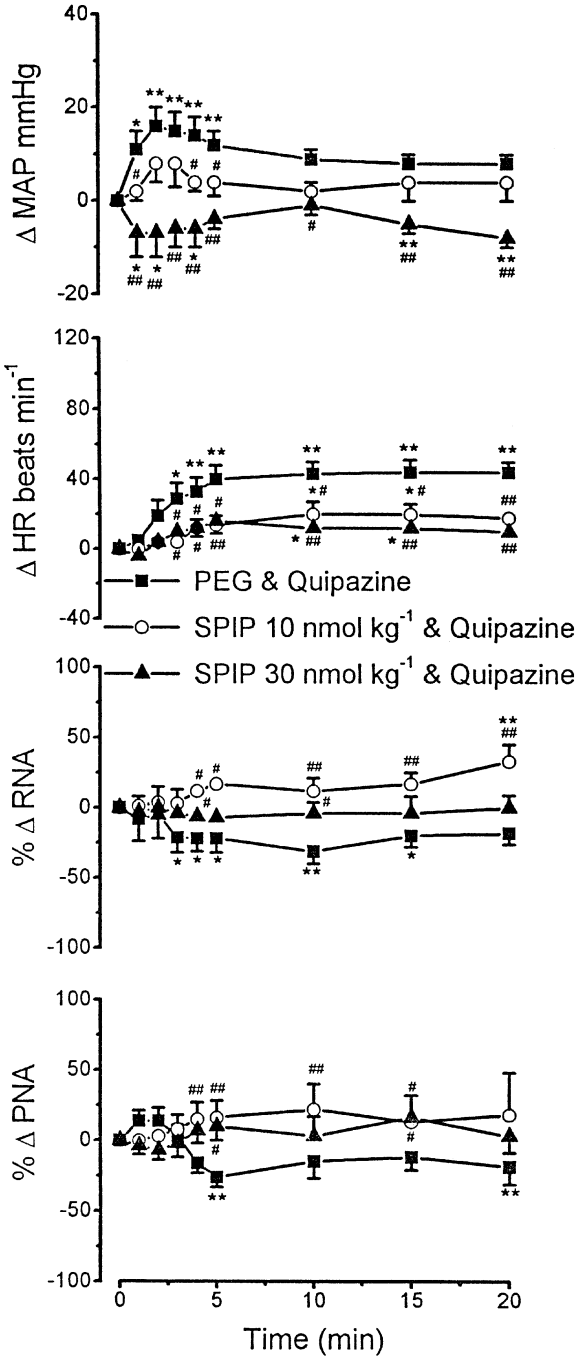

Figure 5 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.): a comparison of the changes from baseline over time (min) caused by quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of 10% PEG (5 μl, i.c.v., $n=8$), quipazine (2 μmol kg^{-1}, i.c.v.,) in the presence of spiperone (SPIP) either 10 nmol kg^{-1} ($n=5$) or 30 nmol kg^{-1} ($n=5$), 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. Changes caused by the PEG & Quipazine and SPIP & Quipazine were compared with PEG control (*; PEG control has not been illustrated for the sake of clarity, see Figure 6) and changes caused by SPIP & Quipazine are compared to PEG & Quipazine (#) using two-way analysis of variance followed by a least significant difference test to compare the means. *# $P<0.05$ and **# $P<0.01$.

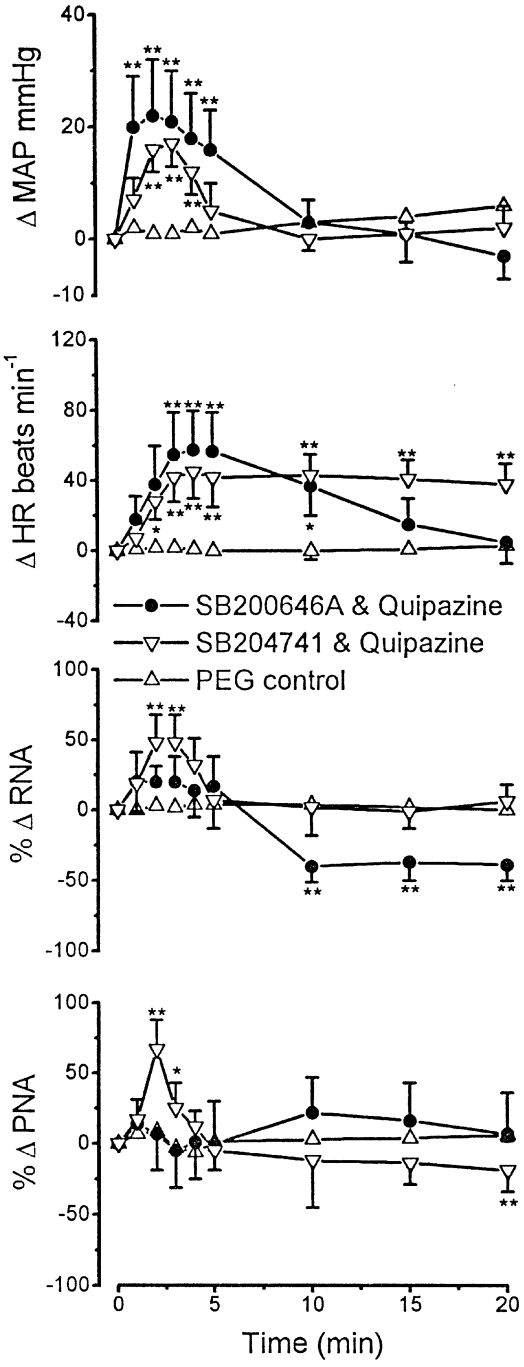

Figure 6 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.): a comparison of the changes from baseline over time (min) caused by quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of SB200646A (300 nmol kg^{-1}, i.c.v., $n=6$) or SB204741 (300 nmol kg^{-1}, i.c.v., $n=6$) and PEG control ($n=10$), 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 the Quipazine & SB200646A or Quipazine & SB204741 were compared with PEG control using two-way analysis of variance followed by a least significant difference test to compare the means. *$P<0.05$ and **$P<0.01$.

min^{-1}, and 111±2 mmHg, 340±12 beats min^{-1} and 35±3 bursts min^{-1}, respectively.

SB200646A and SB204741 pretreatment i.c.v.

SB200646A (300 nmol kg^{-1}, $n=6$) and SB204741 (300 nmol kg^{-1}, $n=6$) failed to have any maintained effect on baseline values.

In the presence of either antagonist, quipazine still caused a similar rise in mean arterial pressure reaching 22±10 mmHg after 2 min and 17±4 mmHg after 3 min, respectively and a tachycardia of 55±22 beats min^{-1} and 48±20 beats min^{-1} after 4 min respectively. However, these changes were now not associated with any initial renal sympathoinhibition (Figure 6). In SB 200646A pretreated rats quipazine caused a delayed (10 min) and significant sympathoinhibition of −40±11% which was not associated with a fall in mean arterial pressure or a bradycardia, although both variables had begun to decline. Conversely in SB 204741 pretreated rats, quipazine caused an initial significant sympathoexcitation which reached a maximum of 48±12% after 2 min. This was also associated with a significant increase in phrenic nerve activity of 67±24% after 2 min. Traces from one of these experiments are shown in Figure 2b. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection in SB 200646A and SB 204741 pretreated rats were

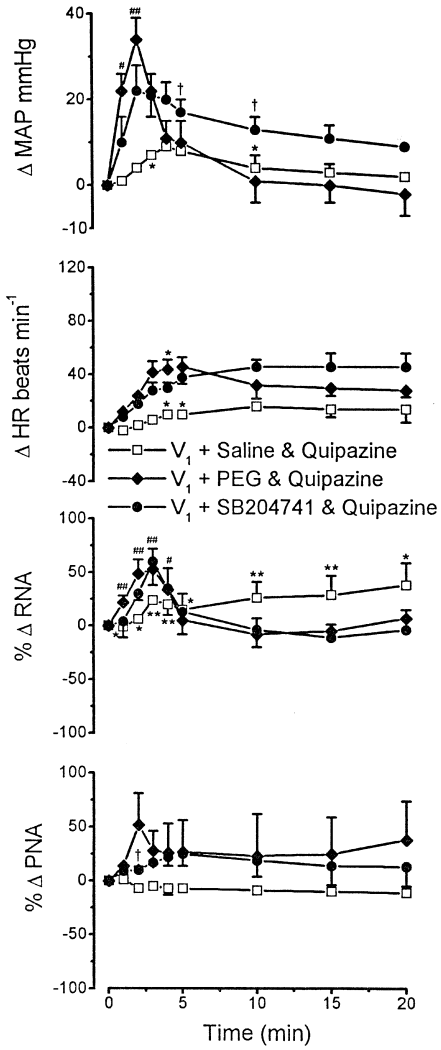

Figure 7 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.) and the vasopressin V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP (V_1, 30 μg kg^{-1}): a comparison of the changes from baseline over time (min) caused by quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of saline (5 μl, i.c.v., $n=4$), 10% PEG (5 μl, 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. Changes caused by the Saline & Quipazine (*), PEG & Quipazine (#) and SB204741 & Quipazine (†) in the presence of the V_1 receptor antagonist are compared to those observed in unpretreated rats (Saline & Quipazine, atropine/V_1 control and SB204741 and Quipazine; data not shown for the sake of clarity, see Figures 1, 3 and 6) using two-way analysis of variance followed by a least significant difference test to compare the means. *#†$P<0.05$ and **## $P<0.01$.

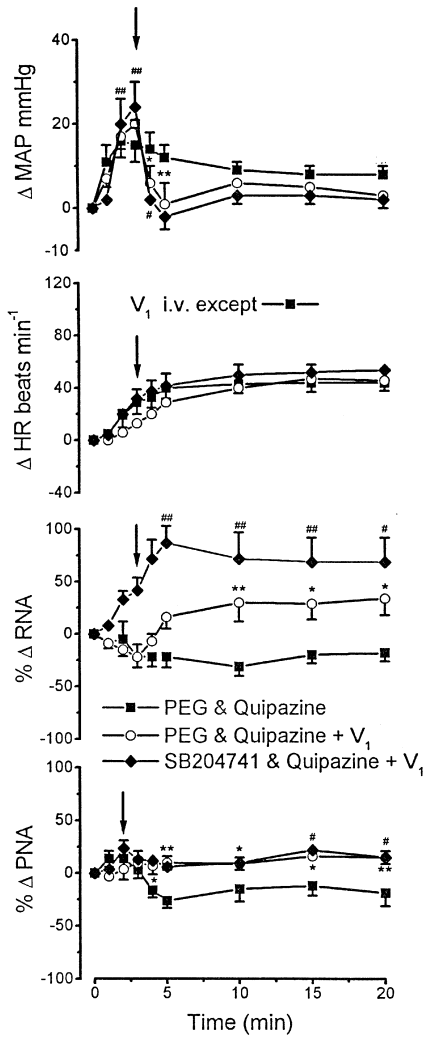

Figure 8 Anaesthetized rats pretreated with BW501C67 (0.1 mg kg^{-1}, i.v.: a comparison of the changes from baseline over time (min) caused by quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of 10% PEG (5 μl, i.c.v. $n=8$) and quipazine (2 μmol kg^{-1}, i.c.v.) in the presence of 10% PEG (5 μl, i.c.v. $n=5$) or SB204741 (300 nmol kg^{-1}, i.c.v. $n=5$) in which vasopressin V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP (V_1, 30 μg kg^{-1}) has been administered (↓) i.v. at the 3 min time point, 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 the PEG & Quipazine + V_1 are compared with PEG & Quipazine (*) while SB204741 & Quipazine + V_1 (#) are compared with SB204741 & Quipazine (data not shown for the sake of clarity, see Figure 6) using two-way analysis of variance followed by a least significant difference test to compare the means. *# $P<0.05$ and **## $P<0.01$.

122±5 mmHg, 334±15 beats min^{-1} and 38±5 bursts min^{-1} and 117±2 mmHg, 316±17 beats min^{-1} and 53±1 bursts min^{-1}, respectively.

Further administration of DOI (n=4) in the presence of SB 204741 evoked a similar response to that observed with quipazine. However, the initial renal sympathoexcitation was followed by a delayed but significant sympathoinhibition of 24±11%, which was maintained for the duration of the experiment. Further the transient increase in phrenic nerve activity observed with DOI alone was now delayed (4 min) reaching 31±18% (data not illustrated). The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection in SB 204741 pretreated rats were 110±4 mmHg, 330±12 beats min^{-1} and 54±3 bursts min^{-1}.

Effect of V_1 receptor antagonist i.v. on quipazine i.c.v. induced changes

Pretreatment Pretreatment with the V_1 receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP 10 μg kg^{-1} (n=5) and 30 μg kg^{-1} (n=14) failed to have any effect on the baseline variables being recorded.

In the presence of 10 μg kg^{-1} of the V_1 receptor antagonist and i.c.v. PEG, quipazine still caused a similar rise in mean arterial pressure of 14±5 mmHg and a tachycardia of 38±7 beats min^{-1}, which were maintained over the course of the experiment. However, rather than renal sympathoinhibition, this was now associated with an immediate, significant but transient, sympathoexcitation (61±5%) returning to baseline after 2 min when compared to atropine/V_1 control (data not illustrated). In the presence of the high dose (30 μg kg^{-1}) of the V_1 receptor antagonist and i.c.v. PEG, quipazine still caused a rise in mean arterial pressure but this was now significantly potentiated (34±5 mmHg *c.f.* 16±5 mmHg quipazine alone) returning to baseline after 10 min. Again the rise in mean arterial pressure was associated with an immediate and significant renal sympathoexcitation rather than inhibition when compared with atropine/V_1 control, reaching a maximum of 58±24% at 3 min and declining to baseline by 5 min. Again the quipazine-evoked tachycardia was unaffected, while phrenic nerve activity remained unchanged (Figure 7). Traces from one of these experiments are shown in Figure 2c. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection in d(CH$_2$)$_5$Tyr(Me)AVP 10 and 30 μg kg^{-1} pretreated rats were 104±4 mmHg, 360±12 beats min^{-1} and 42±3 bursts min^{-1} and 112±4 mmHg, 360±8 beats min^{-1} and 40±1 bursts min^{-1}, respectively.

In the presence of a high dose (30 μg kg^{-1}) of the V_1 receptor antagonist plus saline (5 μl, i.c.v., n=4) instead of PEG, the quipazine-induced pressor response was delayed by 1 min and the size of this rise was significantly reduced (11±4 mmHg *c.f.* 20±7 mmHg). However, this was not associated with an initial sympathoinhibition (see Figure 7) but an immediate, significant, excitation in renal nerve activity of 32±17% by 3 min. In addition, quipazine now evoked a change in heart rate, causing a significant tachycardia of 13±4 beat min^{-1}. Phrenic nerve activity was again unaffected. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection were respectively 104±4 mmHg, 350±8 beats min^{-1} and 40±3 bursts min^{-1}.

At the peak pressor response Injection i.v. of (CH$_2$)$_5$Tyr(Me)AVP (30 μg kg^{-1}, n=5) at 3 min after quipazine (PEG pretreated) caused an immediate fall in mean arterial pressure to baseline values (Figure 8), and the inhibition of renal nerve activity was reversed reaching a maximum increase of 22±18% 8 min later. This increase in renal nerve activity was not significant when compared with PEG control. The quipazine-induced tachycardia was unaffected by injection of the V_1 receptor antagonist while the fall in phrenic nerve activity was inhibited at the 5 min time point. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection were 110±4 mmHg, 345±21 beats min^{-1} and 45±4 bursts min^{-1}.

Effect of V_1 receptor antagonist i.v. on quipazine i.c.v. induced changes in the presence of i.c.v. SB204741

Pretreatment The effect of quipazine in the presence of the high dose (30 μg kg^{-1}, n=5) of the V_1 receptor antagonist and SB204741 (300 nmol kg^{-1}) was similar to that obtained in animals just pretreated with SB204741 alone (Figures 6 and 7). The only slight differences were that there was a slight delay in the decline of the pressor response returning to baseline at 15 min instead of 10 min, and a blocking of the increase in phrenic nerve activity at the 5 min time point. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection were 112±4 mmHg, 360±8 beats min^{-1} and 40±1 bursts min^{-1}.

At the peak pressor response Injection i.v. of the V_1 receptor antagonist (30 μg kg^{-1}, n=5) at 3 min after quipazine also caused an immediate fall in mean arterial pressure to baseline values (Figure 8). This was associated with a parallel potentiation of the renal sympathoexcitation which increased to 87±5% by 5 min and was maintained over the duration of the experiment. The V_1 receptor antagonist affected neither the tachycardia nor phrenic nerve activity. The baseline values for mean arterial pressure, heart rate and phrenic nerve burst rate before quipazine injection were 109±3 mmHg, 340±18 beats min^{-1} and 43±3 bursts min^{-1}.

Discussion

Involvement of central 5-HT$_2$ receptors

Injection of the 5-HT$_2$ receptor agonist quipazine (Hong *et al.*, 1969; Alper & Snider, 1987; Vayssettes-Courchay *et al.*, 1990) in the present experiments into the third ventricle of neuromuscular blocked anaesthetized rats caused a dose related increase in arterial blood pressure, renal sympathoinhibition but no change in heart rate. However, in the rats which were pretreated i.c.v. with PEG, the vehicle used for the antagonist studies, the pressor response to quipazine had a slightly earlier onset and was associated with a significantly smaller decrease in renal sympathetic nerve activity and a tachycardia. This is the first report of the effect of pretreatment i.c.v. with PEG on quipazine (i.c.v.) evoked responses. As these effects of quipazine occurred in rats pretreated i.v. with BW501C67, a peripherally acting 5-HT$_2$ receptor antagonist (Mawson & Whittington, 1970; Fuller *et al.*, 1986), and as the same dose of quipazine given i.v. failed to have any effect on arterial blood pressure in these rats, this indicates that quipazine is acting within the brain to cause these cardiovascular effects. These effects of quipazine are blocked by pretreatment with the 5-HT$_2$ receptor antagonist cinanserin (Rubin *et al.*, 1964), given i.c.v. at a dose reported *in vivo* to block the 5-HT$_2$ receptor

agonist action of 5-HT and DOI (Anderson *et al*., 1992; 1995), and further, the 5-HT$_2$ receptor agonist DOI (see Hoyer & Fozard, 1991) when given i.c.v. was found to cause similar cardiovascular effects to those of quipazine in the present experiments. These combined observations indicate that the cardiovascular effects observed with quipazine, and by inference DOI, are due to activation of central 5-HT$_2$ receptors. The present observations are similar to those previously reported in conscious rats for quipazine, in which the induced rise in arterial blood pressure was shown to be completely blocked by the centrally acting 5-HT$_2$ receptor antagonist LY 53857 and only partially blocked by the peripherally acting 5-HT$_2$ receptor antagonist xylamidine (Zink *et al*., 1990). In these conscious rats, as in the saline pretreated rats in the present study, quipazine failed to affect heart rate. Further, i.c.v. administration of DOI in conscious rats has been shown to increase arterial blood pressure by activation of central 5-HT$_2$ receptors (Dedeoğlu & Fisher, 1991). The differences between the effects of quipazine in PEG and saline pretreated rats may be presumed to be one of access to an additional site/s (see later). Furthermore, the ability of atropine pretreatment to potentiate the tachycardia and rise in arterial blood pressure suggests that quipazine is also capable of inducing an increase in vagal tone to the heart. However, whether this is a direct effect on central pathways involved in the regulation of cardiac vagal drive and/or due to activation of the baroreceptor reflex caused by the release of vasopressin remains to be determined. With respect to the second possibility, the level of renal sympathoinhibition, if due to a baroreceptor reflex in response to the peripherally mediated rise in arterial pressure caused by vasopressin release, would have been expected to be larger as the pressor response had been potentiated in the presence of atropine. This potentiation could also be, in some part, due to atropine methonitrate interfering with the ability of vasopressin to modify the central sympathoexcitation also evoked by quipazine (see later and Figure 9). Overall, the cardiovascular effects evoked by i.c.v. administration of quipazine, and by inference DOI, are due to activation of central 5-HT$_2$ receptors. Further, the 5-HT$_3$ receptor agonist actions of quipazine (Vayssettes-Courchay *et al*., 1990) do not seem to play a role in central cardiovascular actions when it is given i.c.v.

The role of 5-HT$_2$ receptor subtypes

Spiperone, a highly selective 5-HT$_{2A}$ receptor antagonist (see Bonhaus *et al*., 1995), which has approximately ten times higher affinity than cinanserin at 5-HT$_{2A}$ receptors (see Hoyer, 1991; Prins *et al*., 1997), attenuated the pressor response and the tachycardia caused by quipazine. In fact, at a dose ten times less than cinanserin, spiperone reversed the rise in arterial pressure to a fall. The quipazine-evoked renal sympathoinhibition was attenuated by both doses of spiperone, although interestingly, in the presence of the low dose of spiperone, nerve activity tended to increase. However, quipazine in the presence of SB200646A, a selective 5-HT$_{2B/2C}$ receptor antagonist (Kennett *et al*.,

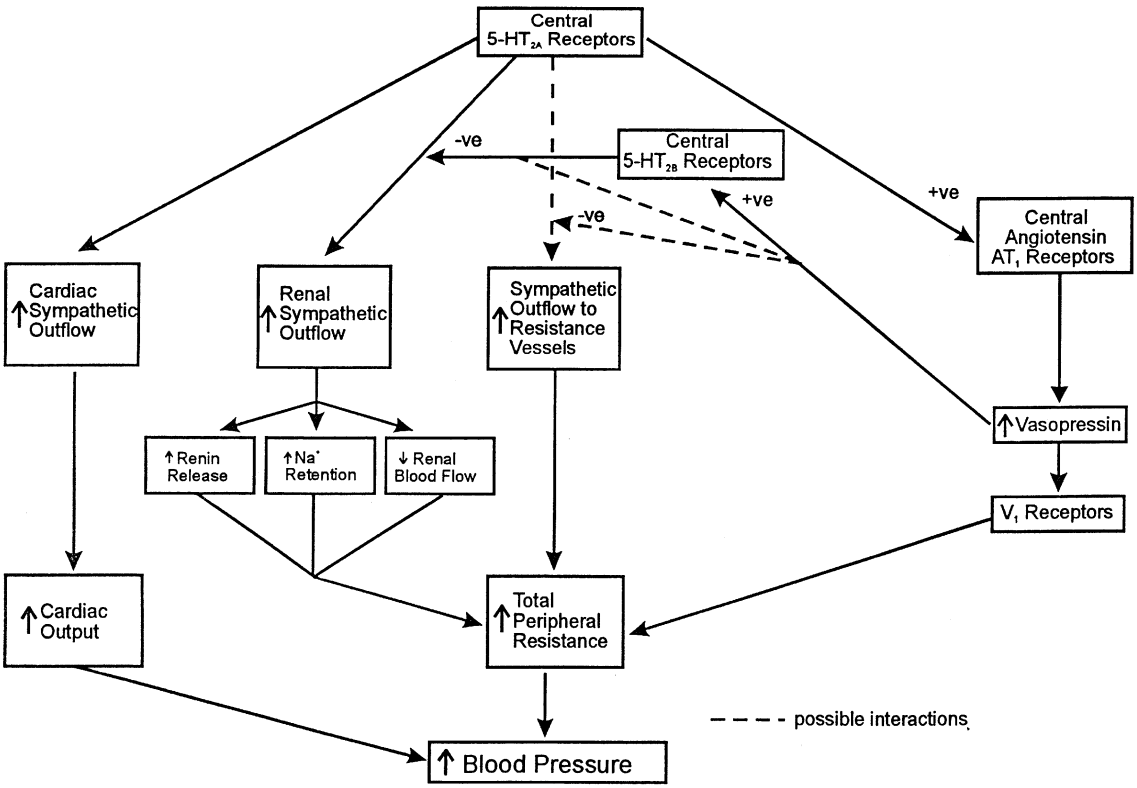

Figure 9 A summary of the effects of i.c.v. quipazine in anaesthetized, neuromuscular blocked and artificially respired male rats. Activation of central 5-HT$_{2A}$ receptors by quipazine causes sympathoexcitation and the release of vasopressin. The released vasopressin modifies this sympathoexcitatory action of quipazine and this modification involves the indirect activation of central 5-HT$_{2B}$ receptors. It should be pointed out that there is another complicating factor; that of baroreceptor reflex-mediated sympathoinhibition in response to the evoked rise in arterial blood pressure. This has not been added to the diagram for the sake of clarity.

1994), and SB204741, a selective 5-HT$_{2B}$ receptor antagonist (Forbes *et al*., 1995), at the same dose as cinanserin, failed to block the quipazine-induced rise in arterial blood pressure. Nevertheless, both drugs abolished the initial renal sympathoinhibition. In the presence of SB204741, quipazine now caused renal sympathoexcitation, which declined to baseline levels by 5 min while after 5 min in the presence of SB200646A quipazine caused sympathoinhibition. The quipazine-induced tachycardia was unaffected by either antagonist, although in the presence of SB200646A the tachycardia was no longer maintained for the duration of the experiment but paralleled the decrease in renal nerve activity that occurred after 5 min. Both of these 5-HT$_2$ receptor antagonists have a very low affinity for 5-HT$_{2A}$ receptors (pK_i of 5; see Bonhaus *et al*., 1995; Forbes *et al*., 1995) and combined with the spiperone data this would indicate that the rises in arterial blood pressure and heart rate evoked by quipazine are due to activation of central 5-HT$_{2A}$ receptors. However, the ability of SB200646A and SB204741 to inhibit the renal sympathoinhibition without affecting the pressor response indicates that this effect involves the activation of 5-HT$_{2B/2C}$ receptors. Furthermore, as SB204741 pretreatment reverses the renal sympathoinhibition to a significant sympathoexcitation, and as SB204741 has a ten times greater affinity for 5-HT$_{2B}$ receptors and 50 times less affinity for 5-HT$_{2C}$ compared with SB200646A, this would indicate that renal sympathoinhibition evoked by quipazine and DOI involves activation of 5-HT$_{2B}$ and not 5-HT$_{2C}$ receptors. Whether the renal sympathoinhibition is induced by quipazine is directly due to activation of 5-HT$_{2B}$ receptors or indirectly by being involved in a feedback response caused by the peripherally induced rise in arterial blood pressure and/or vasopressin release is not clear (see later and Figure 9). Furthermore, the present observations doe not rule out a role for 5-HT$_{2C}$ receptors in the quipazine-induced changes in the cardiovascular variables being measured, although quipazine has a pK_i of 6.2 at 5-HT$_{2C}$ receptors, while it has a pK_i of 6.9 at 5-HT$_{2B}$ receptors (Wainscott *et al*., 1996). Further, the small hypotension and the weak sympathoexcitation observed with quipazine may be mediated by activation of 5-HT$_{2B}$ receptors. In this respect, it has been reported that, in this model (Knowles & Ramage, 1998a), the 5-HT$_{2B}$ receptor agonist BW723C86 (Kennet *et al*., 1996), when given i.c.v., causes a small fall in arterial blood pressure, no change in heart rate and renal sympathoexcitation. This would favour the interpretation that the renal sympathoinhibition is secondary to the quipazine-induced rise in arterial blood pressure caused by vasopressin release (see below and Figure 9) and that this 'feedback effect' of vasopressin involves the action 5-HT$_{2B}$ receptors.

Involvement of vasopressin

Activation of central 5-HT$_2$ receptors involves the release of vasopressin (Anderson *et al*., 1992; Pérgola *et al*., 1993) and the present data indicate that this is mainly mediated by the 5-HT$_{2A}$ receptor subtype. This release of vasopressin could mask other cardiovascular effects by activation of the baroreceptor reflex in response to the peripherally induced rise in arterial blood pressure. However, in the present experiments, pretreatment with d(CH$_2$)$_5$Tyr(Me)AVP, a V$_1$ receptor antagonist, failed to block the pressor effect of i.c.v. quipazine. In fact, in the presence of the high dose of the V$_1$ receptor antagonist, the quipazine-induced pressor response was potentiated, at least in PEG pretreated rats. This could be interpreted as being because quipazine given i.c.v. does not cause the release of vasopressin and thus the pressor response is mediated by another mechanism such as central sympathoexcitation. However, the immediate fall in arterial blood pressure caused by i.v. administration of the V$_1$ receptor antagonist during the peak of the quipazine-induced pressor response and the fact that the V$_1$ receptor antagonist had no effect on baseline arterial blood pressure when given i.v. alone demonstrates that i.c.v. quipazine causes the release of vasopressin. Nonetheless, the fact that pretreatment with a V$_1$ receptor antagonist potentiates the quipazine-induced pressor response and reverses the renal sympathoinhibition to excitation supports the view that quipazine-induced vasopressin release is inhibiting the expected 5-HT$_{2A}$ receptor mediated sympathoexcitation (see Figure 9). As suggested above, the sympathoinhibitory action of the released vasopressin could be indirect by baroreceptor activation and/or by vasopressin itself modifying the function of brain structures involved in cardiovascular regulation (see Berecek, 1993). In this respect, it is interesting that the tachycardia was not potentiated, since if a baroreceptor reflex were masking the 5-HT$_{2A}$ receptor-mediated sympathoexcitation, potentiation of the tachycardia would have been expected. Furthermore, quipazine produces a very similar effect, on the variables being measured, in the presence of the 5-HT$_{2B}$ receptor antagonist SB204741 to that produced in the presence of a V$_1$ receptor antagonist. This would suggest that 5-HT$_{2B}$ receptors are also needed to be activated in addition to 5-HT$_{2A}$ receptors before vasopressin is released in response to i.c.v. quipazine. However, another possible explanation is that when vasopressin acts on brain areas involved in sympathetic regulation it prevents the 5-HT$_{2A}$ receptor mediated sympathoexcitation, and this involves the activation of 5-HT$_{2B}$ receptors indirectly (see Figure 9). The ability of the V$_1$ receptor antagonist, given i.v., at the peak of the quipazine-induced pressor response to cause an immediate fall in arterial blood pressure, in SB204741 pretreated rats, demonstrates that SB204741 does not interfere with the quipazine-induced release of vasopressin. This would support the view that 5-HT$_{2B}$ receptors are not involved in the i.c.v. quipazine-evoked release of vasopressin, but in the ability of the released vasopressin to modify brain areas involved in central cardiovascular regulation. It is worthy of note that when the V$_1$ receptor antagonist is given i.v. at the peak of the quipazine-induced pressor response, in both groups there is an increase in renal nerve activity, although there is no change in heart rate and arterial blood pressure only slightly increases above its original baseline value. This could be interpreted to indicate that vasopressin is at least tonically inhibiting renal sympathetic outflow. If this increase in renal nerve activity were due to baroreceptor activation in response to the induced fall in arterial blood pressure, this would indicate that baroreceptors have been reset upwards. If so it would be expected that this fall in arterial blood pressure would also cause an increase in heart rate, which did not occur.

Therefore the observations in the present experiments favour the view that the vasopressin release, caused by activation of 5-HT$_{2A}$ receptors, is interfering with a sympathoexcitation, also mediated by 5-HT$_{2A}$ receptors, to the kidney and to resistance vessels (see Figure 9). This would be consistent with the observation that, in rats pretreated with the V$_1$ receptor antagonist, the quipazine-induced pressor response was potentiated and the renal sympathoinhibition reversed to excitation. Interestingly,

again the tachycardia was unaffected. However, it is somewhat surprising that, when the V_1 receptor antagonist is given at the peak of the quipazine-induced pressor response, the fall in arterial blood pressure is not transient. It would be expected that if the central sympathoexcitation to resistance vessels was switched back on, similarly to that observed for renal outflow, arterial blood pressure should begin to rise again to a similar level to that before the administration of V_1 receptor antagonist. This suggests that the sensitivity of central mechanisms involved in the control of renal sympathetic outflow by the released vasopressin differs from that of other sympathetic outflows. This difference may involve activation of 5-HT$_{2B}$ receptors. In this respect, preliminary experiments in the present model have reported that activation of central 5-HT$_{2B}$ receptors, causes renal sympathoexcitation and no change in heart rate (Knowles & Ramage, 1998b).

Site of action

The rapid onset of the response when either quipazine or DOI were administered i.c.v. suggests that brain area/s close to the lateral or third ventricle are responsible for the effects produced. Vasopressin release can be induced by i.c.v. angiotensin II and this has been attributed to the activation of angiotensin receptors located in the subfornical organ (see Hartle & Brody 1984; Iovino & Steardo, 1985). This region of the brain is one of the so-called circumventricular organs and does not have a blood brain barrier and is thus ideally suited to detecting peptide hormones (see Dellmann & Simpson, 1979). In addition, this region receives a serotonergic projection from the dorsal and median raphe (Lind, 1986) and more recently it has been shown that vasopressin released by i.c.v. administration of 5-HT is blocked by i.c.v. pretreatment with the angiotensin II antagonist, losartan (Saydoff *et al*., 1996), and also, in the present experimental system, that i.c.v. losartan blocks the quipazine-induced rise in arterial blood pressure but not the tachycardia and reverses the renal sympathoinhibition to excitation (Knowles & Ramage, 1998a). Furthermore, microinjection of 5-HT into the anterior hypothalamus/pre-optic area, another region that contains serotonergic neurones (Steinbusch, 1981), causes an increase in arterial blood pressure with little or no change in heart rate (Smits & Struyker-Boudier, 1976). 5-HT$_2$ receptors have also been identified in the above mentioned areas (Pazos *et al*., 1985) and the 5-HT$_{2B}$ receptor subtype has been found in the lateral septum, which surrounds the subfornical organ, the dorsal hypothalamus and medial amygdala (Duxon *et al*., 1997). Whether quipazine, when given i.c.v., is only acting at one of the sites or more is difficult to assess. However, when comparing the effects of quipazine i.c.v. in the presence of saline with those of PEG, quipazine evokes a larger renal sympathoinhibition and no tachycardia in the former case, although the pressor responses are similar. It should be noted that this latter observation implies that PEG is probably not interfering with the ability of vasopressin to feedback and modify the 5-HT$_{2A}$ receptor-evoked sympathoexcitatory response. Now when this saline group is pretreated with a V_1 receptor antagonist, the tachycardia and renal sympathoexcitation are observed. This is similar to the effects of quipazine in the presence of a V_1 receptor antagonist and PEG, yet the latter effects are significantly smaller. Further, the pressor response induced by quipazine is not blocked by the V_1 receptor antagonist in the saline pretreated group nor is it potentiated as in the PEG pretreated group. Overall these observations suggest that, in the presence of saline, the ability of quipazine to cause central sympathoexcitation, particularly concerning renal sympathetic outflow, is reduced compared to that observed in rats pretreated with PEG. Therefore PEG pretreatment does not seem to change the ability of i.c.v. quipazine to cause vasopressin release nor a pressor response, only to cause renal sympathoexcitation and tachycardia. If PEG pretreatment affects diffusion barriers for quipazine, then these data suggest that the site/s at which quipazine causes central renal sympathoexcitation and heart rate changes is/are not the same at which it causes vasopressin release.

Central respiratory control

The major problem with assessing changes in phrenic nerve activity is that changes in arterial blood pressure will themselves cause changes in phrenic nerve activity. For instance an increase arterial blood pressure will tend to cause a reduction in phrenic nerve activity (see Coleridge *et al*., 1997). If this is taken into account the data suggest that quipazine tends to prevent this expected inhibition of phrenic nerve activity when arterial blood pressure rises and, in the latter stages, inhibits phrenic nerve activity. As these effects are on phrenic nerve bursts size and not burst frequency it is likely that the activation of central 5-HT$_2$ receptors causes an increase in the recruitment of phrenic nerve fibres to each respiratory burst. In the presence of the 5-HT$_{2B}$ receptor antagonist SB204741, although not in the presence of SB200646A, quipazine induced a very large initial rise in phrenic burst size, suggesting the involvement of 5-HT$_{2B}$ receptors in this response. Interestingly there is a tendency for quipazine to cause an increase in phrenic burst size in the presence of the V_1 receptor antagonist. This again suggests that the expected rise in phrenic nerve activity is being inhibited by vasopressin release that there is feedback onto the brain structure/s involved in the control of phrenic nerve activity and that feedback involves the activation of 5-HT$_{2B}$ receptors. In this respect, DOI alone initially caused significantly increased phrenic burst size. The difference between DOI and quipazine in the present experiments could be considered to be one of potency, in that the released vasopressin is not sufficient to suppress this action fully as in the case of quipazine. Therefore activation of 5-HT$_2$ receptors in the areas of the brain accessed by the i.c.v. route is involved in the excitatory control of central respiratory drive.

Conclusion

The present experiments demonstrate that in the rat as in cats, activation of 5-HT$_2$ receptors causes sympathoexcitation, at least at the level of the brain accessed by the i.c.v. route of administration. In addition, the study confirms that activation of 5-HT$_2$ receptors at this level of the brain causes the release of vasopressin. The present experiments also indicate that the 5-HT$_{2A}$ receptor subtype mediates both of these effects. In addition, the cardiovascular effects caused by i.c.v. quipazine and DOI also involve the activation of 5-HT$_{2B}$ receptors. However, detailed interpretation of the data from the present

experiments indicates that the involvement of 5-HT_{2B} receptors in the cardiovascular effects induced by quipazine and DOI is not primarily due to direct activation of these receptors by these agonists. In fact, the data suggest the 5-HT_{2B} receptors are involved in the mechanism by which the released vasopressin interferes with the expected 5-HT_{2A} receptor-mediated sympathoexcitation, at least at the level of renal sympathetic outflow (see Figure 9). Finally, the present observations are the first to indicate that central 5-HT_{2B} receptors are involved in cardiovascular regulation.

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