British Journal of Pharmacology (2008) 153, 1728–1738
 0007–1188/08 $30.00
www.brjpharmacol.org

RESEARCH PAPER

Cardiovascular effects of activation of central α7 and α4β2 nAChRs: a role for vasopressin in anaesthetized rats

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Background and purpose: Central application of nicotine causes the release of vasopressin and affects blood pressure. Involvement of the 5 neuronal nicotinic receptor groups, α2*–α7* in these effects is unknown. The availability of selective agonists for α7 (PSAB-OFP) and α4β2 (TC-2559) nACh receptors allowed their role to be investigated.
Experimental approach: Recordings were made of arterial blood pressure, heart rate and renal sympathetic nerve activity in anaesthetized male rats with neuromuscular blockade and artificial respiration. Effects of the agonists, PSAB-OFP (1–10 μmol kg^{-1}) and TC-2559 (1–10 μmol kg^{-1}) on these variables given intracerebroventricularly (i.c.v.) and intracisternally (i.c.) in the presence or absence of the antagonists, DhβE (10 μmol kg^{-1}) and MLA (0.5 μmol kg^{-1}), for the appropriate nicotinic receptor subtypes, respectively, and a V_1 receptor antagonist, given i.v. or centrally, were investigated.
Key results: Both agonists given i.c.v. caused a delayed rise in blood pressure and renal nerve activity which could be blocked only with the appropriate antagonist. The agonists had an earlier onset of action when given i.c., favouring the brainstem as the major site of action. The effects of these agonists were also attenuated by the V_1 receptor antagonist given i.v. and blocked when this antagonist was given centrally. Antagonists had no effect on baseline variables.
Conclusions and implications: Activation of α4β2 and α7 receptors in the brainstem is mainly responsible for the cardiovascular effects of activating these receptors, which have a similar profile of action. These actions, although independent, are mediated by the central release of vasopressin.
British Journal of Pharmacology (2008) **153,** 1728–1738; doi:10.1038/bjp.2008.47; published online 25 February 2008

Keywords: blood pressure; nicotinic ACh receptor; sympathetic nerve activity; vasopressin V_1 receptors; PSAB-OFP; TC-2559; vasopressin; α4β2 nAChRs; α7 nAChRs

Abbreviations: DhβE, dihydro-β-erythroidine; HR, heart rate; i.c., intracisternal; i.c.v., intracerebroventricular; i.v., intravenous; Int, integrated; MAP, mean arterial blood pressure; MLA, methyllycaconitine citrate; nAChRs, nicotinic acetylcholine receptors; PSAB-OFP, (R)-N-(1-Azabicyclo(2.2.2)oct-3-yl)(5-(2-pyridyl)thiophene-2-carboxamide; RNA, renal sympathetic nerve activity; TC-2559, (E)-N-methyl-4-(3-(5-ethoxypyridin)yl)-3-buten-1-amine

Introduction

It has long been known that activation of nicotine receptors in the brain causes the release of vasopressin (Burn *et al*., 1945; Bisset *et al*., 1975). However, which of the possible five neuronal nicotinic receptor groups, α2*–α7* (see Colquhoun *et al*., 2003; Alexander *et al*., 2007), are involved has yet to be determined. Recently, two compounds, PSAB-OFP ((R)-*N*-(1-Azabicyclo(2.2.2)oct-3-yl)(5-(2-pyridyl)thiophene-2-carboxamide) (Astra IV; Broad *et al*., 2002) and TC-2559 ((E)-*N*-methyl-4-(3-(5-ethoxypyridin)yl)-3-buten-1-amine) (Bencherif *et al*., 2000; Chen *et al*., 2003; Yang *et al*., 2006) have been shown to be selective for the two most predominant neuronal nACh receptors in the human and rat central nervous system, the α7 and α4β2 receptors, respectively. In addition, two selective antagonists are also available, methyllycaconitine citrate (MLA) for α7 receptors (Yum *et al*., 1996) and dihydro-β-erythroidine (DhβE) for the α4β2 (Eaton *et al*., 2003), which can be used to confirm that the effects of the two agonists are mediated by the particular nicotinic acetylcholine receptor (nAChR) subtype for which they show selectivity. Thus, the present experiments were carried out to

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Received 31 October 2007; revised 10 January 2008; accepted 22 January 2008; published online 25 February 2008

determine the effects of these agonists, administered centrally either by intracerebroventricular (i.c.v.) or intracisternal (i.c.) injection on mean arterial blood pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RNA). In addition, the involvement of central and/or peripheral vasopressin release in these cardiovascular effects was also determined by carrying out the above experiments in the presence and absence of a selective vasopressin V_1 antagonist given centrally (i.c.v. or i.c.) and peripherally (i.v.). Preliminary communication of some of these data has previously been given (Moore *et al*., 2004a, b, 2007).

Methods

All the experiments were carried out under the Animals (Scientific Procedures) Act 1986. At the end of each experiment, all animals were humanely killed by an overdose of sodium pentobarbital (i.v.).

Experiments were performed on 158 male Sprague–Dawley rats (250–350 g). Anaesthesia was induced by isoflurane (2.5% in oxygen) and maintained with α-chloralose (100 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 right 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 HR was derived electronically from the blood pressure signal. The left jugular vein was cannulated for drug administration, and the trachea was also cannulated. Body temperature was monitored by a rectal probe and maintained at 36–38 °C with a Homeothermic Blanket Control Unit (Harvard). The animals were artificially ventilated (rate 50 strokes min^{-1}, stroke volume 8 ml kg^{-1}) with oxygen enriched room air by the use of a positive pressure pump (Harvard Rodent Ventilator 683) and neuromuscular block was produced with decamethonium (3 mg kg^{-1}, i.v.). It should be noted that decamethonium is highly charged and is thus unlikely to cross the blood–brain barrier. Blood samples were taken from a T-piece on the carotid arterial cannula, and the blood gases and pH were monitored with a Corning 238 pH/blood gas analyser. Blood gases were maintained between 100–130 mm Hg PO_2, 40–50 mm Hg PCO_2 and pH 7.3–7.4. Adjustments of the respiratory pump volume and/or injection of sodium bicarbonate (1 M) were made as necessary to maintain blood gas and pH balance. Once ventilated, the animals were infused (6 ml kg^{-1} h^{-1}) into the jugular vein with a solution comprising 10 ml plasma substitute (Gelofusine), 10 ml distilled water, 4 mg glucose, 168 mg sodium bicarbonate and 10 mg decamethonium. This was to prevent the development of non-respiratory acidosis and to maintain blood volume and neuromuscular blockade. During neuromuscular blockade, the depth of anaesthesia was assessed by monitoring the stability of arterial blood pressure and HR and the cardiovascular responses to paw pinch. In all the experiments, the arterial blood pressure and HR were found to be unresponsive to paw pinch.

Cannulation of the lateral cerebral ventricle and cisterna magna
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 coordinates used from the bregma were 1.5 mm lateral, 1 mm caudal and 4 mm ventral. For the cisterna magna, the atlanto-occipital membrane was exposed. A stainless steel guide cannula (23 gauge) was inserted perpendicularly into the membrane to the depth of its bevel. Drugs and vehicle solution were administered through an i.c. injection cannula (28 gauge) attached by a length of polythene tubing to a 25 μl syringe (Hamilton). Successful cannulation was verified by the filling of the stainless steel guide cannula with cerebrospinal fluid and at the end of the experiment, by the administration of 5 μl of 2% pontamine sky blue dye.

Recording of renal sympathetic nerve activity
The left kidney was exposed by a retroperitoneal approach and was deflected laterally to reveal the renal artery and nerve. The nerve was cleared of connective tissue and positioned on a bipolar silver hook electrode. The RNA was amplified (Digitimer NL104), filtered (Digitimer NL125, frequency band 100–500 Hz) and quantified by integrating the signal above the background noise over 5 sec with a solid state integrator (Medical Electronics workshop, Royal Free Hospital, see Anderson *et al*., 1992). At the end of the experiment, 20 mg of sodium pentobarbital (per animal) was used to reduce nerve activity to zero to validate the integrator threshold. At the beginning of each experiment, the baroreceptor reflex response was tested by observing whether RNA and HR 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 protocol
The preparation was allowed to stabilize for 20 min before the administration of 5 μl saline (i.c.v. and i.c.; saline flush). Ten minutes after the initial saline flush, a single dose of drug or saline control was given i.c.v. or i.c. and the response was followed for 20 min. In antagonist studies, antagonist or saline was administered i.c.v. 3 min before injection of test drug or saline (i.c.v.) and the response was then followed for 20 min. For the V_1 receptor antagonist, d$(CH_2)_5$Tyr(Me)AVP, experiments in the i.v. studies the antagonist was given 5 min after the initial saline flush (i.c.v. or i.c.) and saline or agonist (i.c.v. or i.c.) 10 min later; the response was followed for 20 min. In another set of experiments, again the preparation was allowed to stabilize for 20 min before flushing with saline (5 μl) (i.c.v. or i.c.) and then 10 min later saline or V_1 receptor antagonist was given i.c.v. or i.c. followed 3 min later by saline or agonist (i.c.v. or i.c.) and the response followed for at least 20 min.

Analysis of results

Baseline values were taken 1 min before the addition of the drug or vehicle. All results are expressed as changes from

baseline values. Nerve activity was measured as the average of the integrated values over 10 s at 1 min intervals for the first 10 min and then as an average over 1 min at 5 min intervals in arbitrary units, and was expressed as the percentage change from baseline. Changes in mean blood pressure, HR and RNA caused by the test drug were compared to time-matched vehicle controls using two-way analysis of variance (ANOVA) and were subsequently analysed using the least significant difference test (see Sokal and Rohlf, 1969). All values are expressed as the mean ± s.e.m.; differences in the mean were taken as significant when $P<0.05$.

Drugs and solutions

Drugs were obtained from the following sources: α-chloralose; sodium nitroprusside; decamethonium bromide, sodium bicarbonate, (Arg^8)-vasopressin from Sigma Chemical Co., Poole, Dorset, UK; noradrenaline acid tartrate from Winthrop, Guildford, Surrey, UK; isoflurane from Abbott Labs Ltd, Queenborough, Kent, UK; Gelofusine from Braun Medical Ltd, Aylesbury, Bucks, UK; sodium pentobarbital (Pentoject) from Animalcare Ltd, York, UK. The following were gifts from Eli Lilly Co.: PSAB-OFP and TC-2559; (β-mercapto-β, β-cyclopentamethylenepropionyl^1,O-Me-Tyr^2,Arg^8)-vasopressin ($d(CH_2)_5$Tyr(Me)AVP); DhβE; and MLA. Drugs were dissolved in 0.9% w/v saline. Solutions were administered i.c.v. and i.c., in a volume of 5 μl over a 20 sec period.

Results

Saline controls

In saline (i.c.v. and i.c.)-pre-treated animals, saline injected i.c.v. and i.c. (5 μl; $n=5$; saline control) had no effect on MAP, HR or RNA, and these variables remained stable for the duration of the experiment (see Figures 1, 2, 4 and 5). The baseline values for MAP and HR before saline i.c.v. ($n=5$) and i.c. ($n=5$) pre-treatment were 137 ± 14 mm Hg and 380 ± 27 beats min^{-1} and 112 ± 13 mm Hg and 385 ± 32 beats min^{-1}, respectively. In saline controls (i.c.v. $n=5$) for antagonist studies, saline was given 3 min after the antagonist and again had no effect in any of the variables being recorded. The baseline values for MAP and HR before initial saline injection in the controls were 113 ± 14 mm Hg and 393 ± 51 beats min^{-1}.

Effects of the α7 receptor agonist PSAB-OFP given centrally (i.c.v. and i.c.)

PSAB-OFP 1 μmol kg^{-1} (i.c.v.; $n=5$) evoked a significant increase (of 34 ± 7%) in RNA at 6 min, but without any change in MAP or HR, whereas 3 μmol kg^{-1} ($n=5$) evoked a larger increase in RNA (126 ± 23%) by 8 min and maximum rise of MAP (23 ± 6 mm Hg) by 5 min; again there was no change in HR (see Figures 1a and 2a). Similarly, the highest dose of 10 μmol kg^{-1} ($n=5$) evoked an even larger increase in

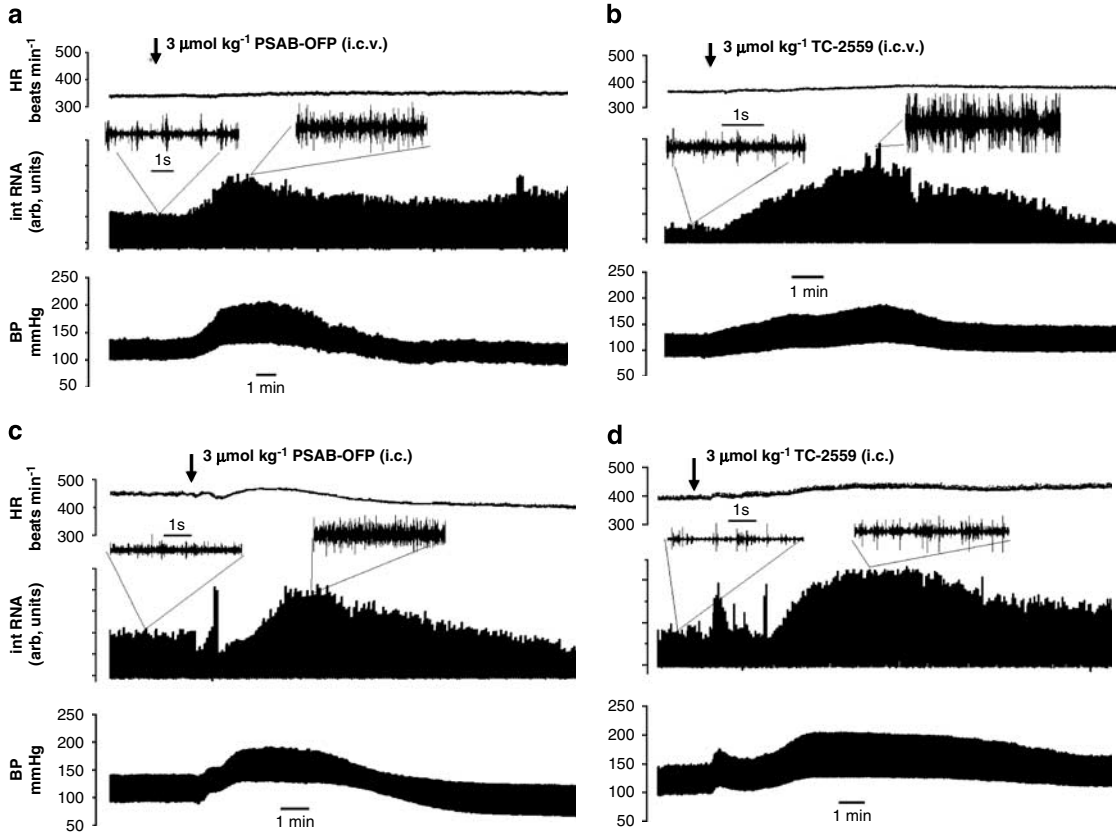

Figure 1 Traces showing the effects of 3 μmol kg^{-1} of (**a**) PSAB-OFP i.c.v. (**b**) TC-2559 i.c.v. (**c**) PSAB-OFP i.c. and (**d**) TC-2559 i.c. on blood pressure (BP), integrated renal nerve activity (Int RNA) and heart rate (HR) in anaesthetized artificially ventilated and neuromuscular blocked rats.

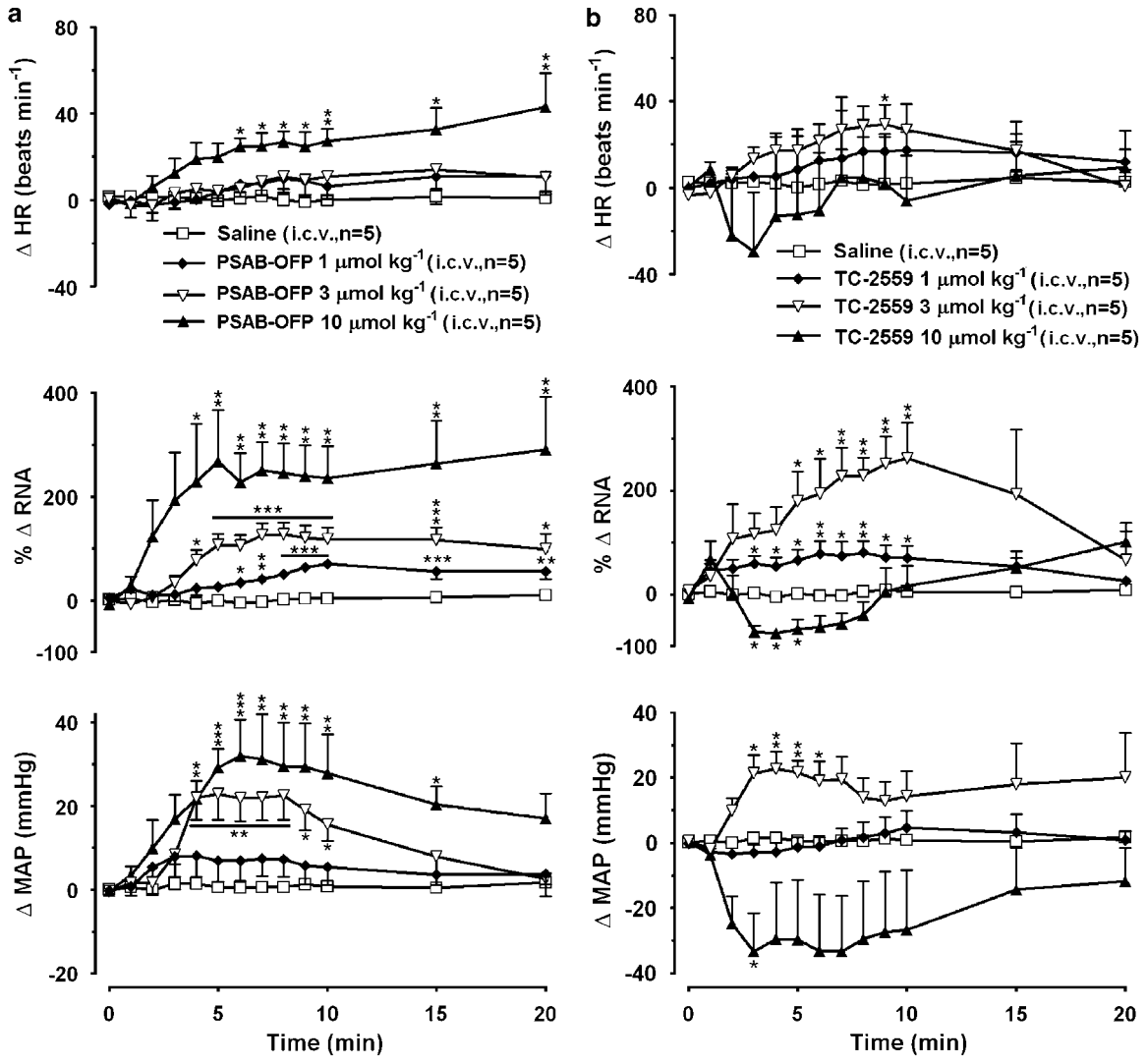

Figure 2 Anaesthetized artificially ventilated and neuromuscular blocked rats: a comparison of the changes (Δ) from baseline values over time (min) caused by three doses of (**a**) PSAB-OFP (1, 3 and 10 μmol kg^{-1}, i.c.v., $n=5$) and (**b**) TC-2559 (1, 3 and 10 μmol kg^{-1}, i.c.v., $n=5$) on mean arterial blood pressure (MAP), renal nerve activity (RNA) and heart rate (HR). Each point represents the mean value and the vertical lines show s.e.m. Changes caused by PSAB-OFP and TC-2559 were compared to saline using two-way ANOVA followed by the least significant difference test to compare the means. *$P<0.05$, **$P<0.01$ and ***$P<0.001$. ANOVA, analysis of variance.

RNA, 290 ± 101% by 5 min, and a rise in MAP, of 32 ± 9 mm Hg after 6 min (Figure 2a). Furthermore, a tachycardia was now observed after 6 min reaching a maximum of 43 ± 16 beats min^{-1} by 20 min. For all doses, the rise in MAP started to decline after about 10 min, but RNA remained elevated.

Baseline values for MAP and HR in the three groups were 122 ± 7 mm Hg and 362 ± 44 beats min^{-1}, 124 ± 8 mm Hg and 356 ± 23 beats min^{-1} and 126 ± 22 mm Hg and 350 ± 23 beats min^{-1}, respectively.

Intracisternal administration of the middle dose (3 μmol kg^{-1}, $n=5$; Figures 1c and 3a) of PSAB-OFP evoked a similar profile of effects to that of i.c.v. administration; however, the onset in the rise in MAP occurred significantly earlier, that is, after 2 min (after i.c.v.injection, it took 4 min, see Figures 1c and 3a), reaching a maximum of 38 ± 8 mm Hg by 4 min.

Baseline values for MAP and HR were 111 ± 11 mm Hg and 387 ± 65 beats min^{-1}.

Effects of the α7 receptor antagonist methyllycaconitine and the α4β2 receptor antagonist dihydro-β-erythroidine i.c.v. on PSAB-OFP i.c.v.

In the presence of MLA (0.5 μmol kg^{-1}; i.c.v.; $n=5$), the effects of PSAB-OFP (3 μmol kg^{-1}; i.c.v.; $n=5$) were blocked (Figure 3a), although there was a tendency of MAP to slowly decrease. However, in the presence of DhβE (10 μmol kg^{-1}; i.c.v.), the effects of PSAB-OFP (3 μmol kg^{-1}, $n=5$) were unaltered, although the rise in MAP was faster, although not significantly (Figure 3a). Neither MLA nor DhβE had any effect on baseline values.

Baseline values for MAP and HR were 114 ± 13 mm Hg and 363 ± 33 beats min^{-1}, 114 ± 13 mm Hg and 363 ± 33

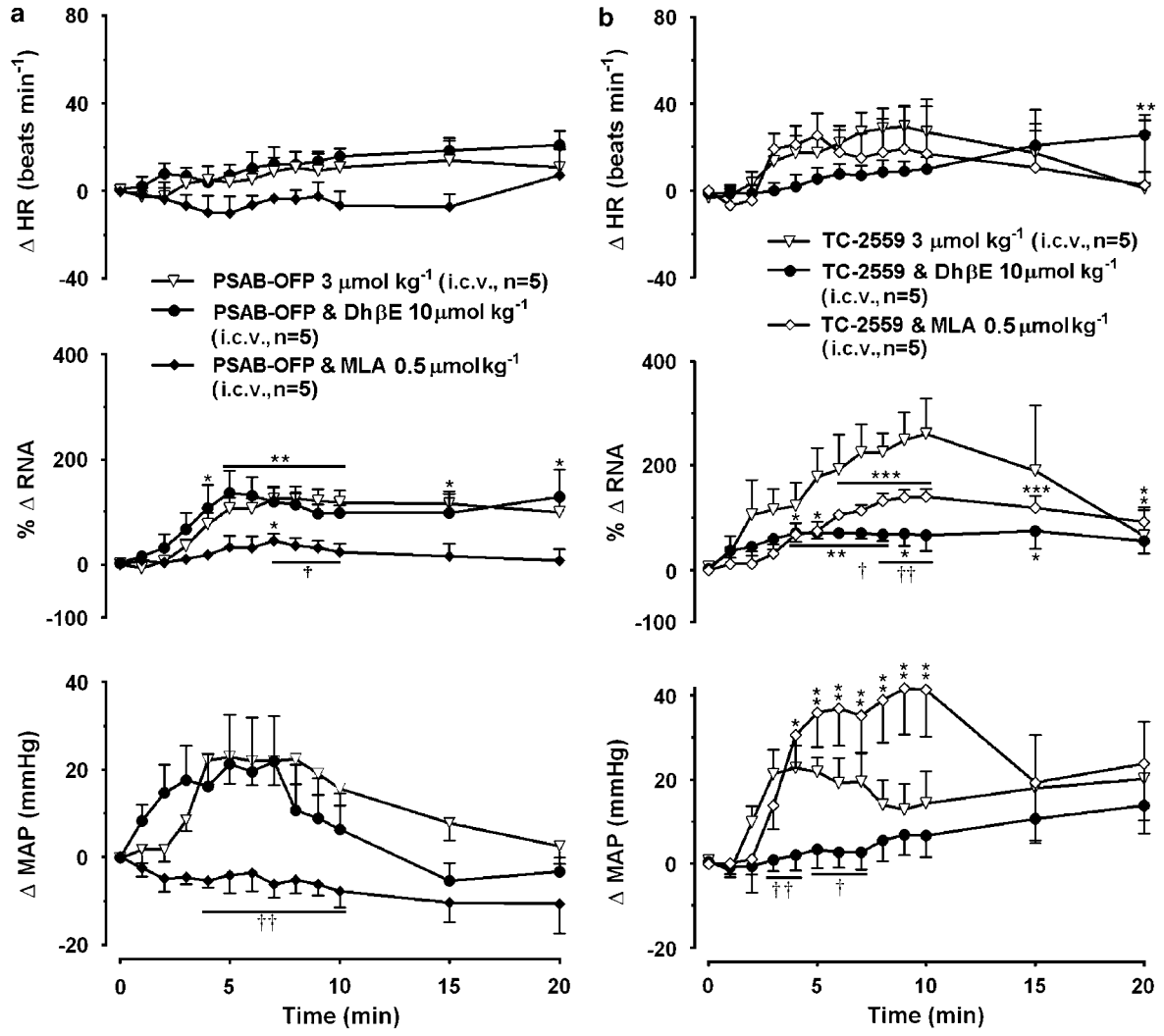

Figure 3 Anaesthetized artificially ventilated and neuromuscular blocked rats: a comparison of the changes (Δ) from baseline values over time (min) caused by (**a**) PSAB-OFP (3 μmol kg^{-1}, i.c.v., $n=5$) in the presence of DhβE (10 μmol kg^{-1}, i.c.v., $n=5$) or MLA (0.5 μmol kg^{-1}, i.c.v., $n=5$) and (**b**) by TC-2559 (3 μmol kg^{-1}, i.c.v., $n=5$) in the presence of DhβE (10 μmol kg^{-1}, i.c.v., $n=5$) or MLA (0.5 μmol kg^{-1}, i.c.v., $n=5$) in mean arterial blood pressure (MAP), renal nerve activity (RNA) and heart rate (HR). Each point represents the mean value and the vertical lines show s.e.m. Changes caused by PSAB-OFP and TC-2559 pre-treated with antagonists are compared to antagonist controls (*data not shown for the sake of clarity) and changes caused by PSAB-OFP (†) pre-treated with DhβE compared with PSAB-OFP alone and TC-2559 (†) pre-treated with DhβE compared with TC-2559 alone by using two-way ANOVA followed by the least significant difference test to compare the means. The bar above or below points indicates that those points have the same level of *P*. *,$^{\dagger}P<0.05$, **,$^{\dagger\dagger}P<0.01$ and ***$P<0.001$. ANOVA, analysis of variance.

beats min^{-1} and 103 ± 9 mm Hg and 377 ± 43 beats min^{-1}, respectively.

Effects of the α4β2 receptor agonist TC-2559 given centrally (i.c.v. and i.c.)

The lowest dose (1 μmol kg^{-1}; $n=5$) of TC-2559 (i.c.v.) had similar effects to PSAB-OFP, only evoking a significant rise in RNA. However, the onset was earlier, after 3 min (with PSAB-OFP, it took 6 min) and reached its maximum of 79 ± 21% at 8 min (see Figure 1b and 2b). The intermediate dose (3 μmol kg^{-1}; $n=5$) caused a very large rise in RNA reaching a maximum by 9 min of 249 ± 53%; however, due to large variability this was not significant until after 5 min. This was now associated, as for the intermediate dose of PSAB-OFP, with a significant rise in MAP of 21 ± 6 mm Hg after 3 min (Figure 2b). Again this was associated with no overall change in HR apart from a significant increase of 29 ± 9 beats min^{-1} at 9 min (Figure 2b). However, the highest dose (10 μmol kg^{-1} ($n=5$) differed completely from PSAB-OFP and the lower doses of TC-2559, evoking a fall in MAP and RNA, reaching a maximum of −34 ± 12 mm Hg and −75 ± 10% by 3 min. The effects on blood pressure were very variable, only being significant at the 3 min time point, as were the effects on HR, although the fall in HR was never significant (Figure 2b).

Baseline values for MAP and HR in the three groups were 120 ± 17 mm Hg and 368 ± 54 beats min^{-1}, 113 ± 16 mm Hg and 366 ± 34 beats min^{-1} and 135 ± 19 mm Hg and 380 ± 14 beats min^{-1}, respectively.

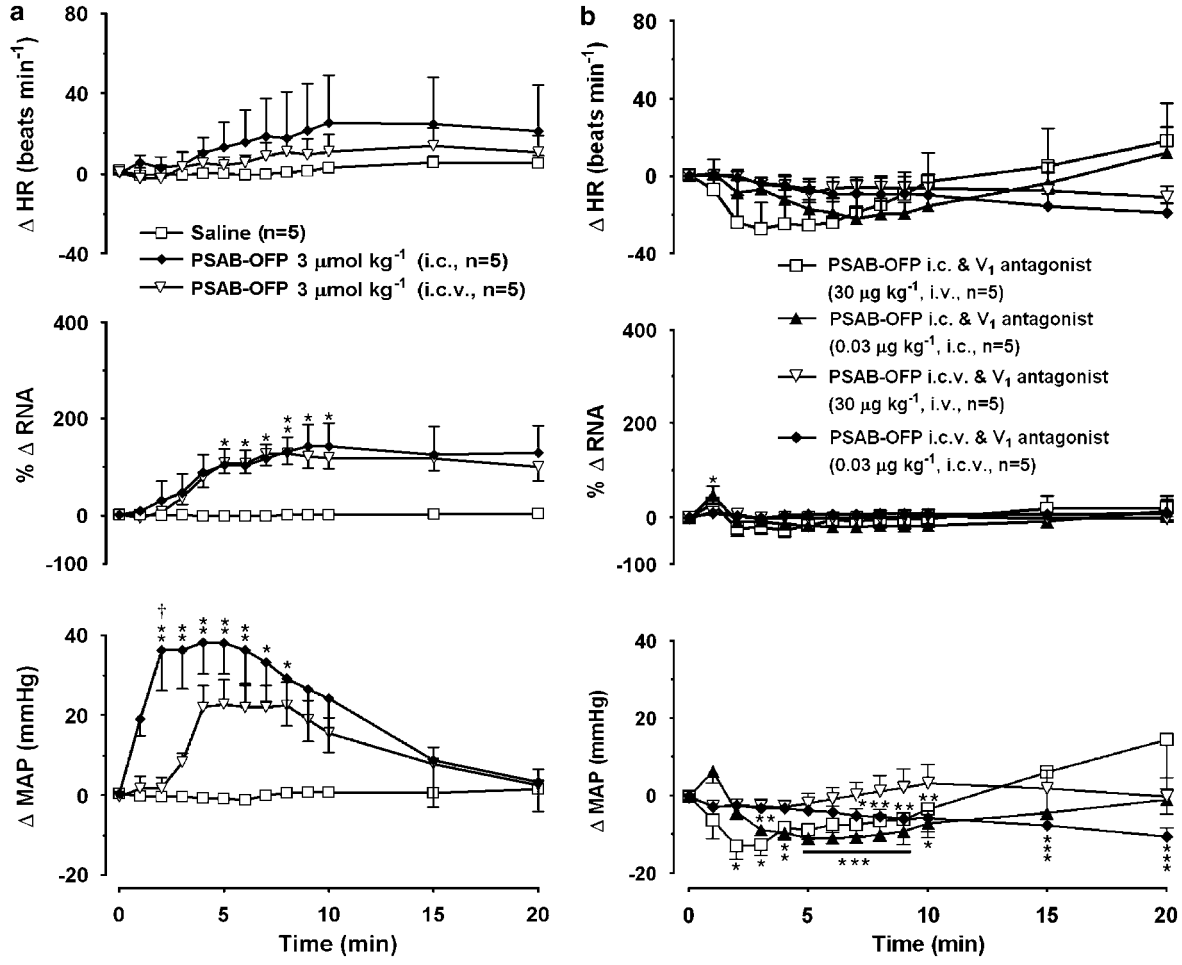

Figure 4 Anaesthetized artificially ventilated and neuromuscular blocked rats: a comparison of the changes (Δ) from baseline values over time (min) caused by (**a**) PSAB-OFP (3 μmol kg^{-1}) given i.c. and i.c.v. and saline (5 μl. i.c.) and (**b**) in the presence of V_1 receptor antagonist ($d(CH_2)_5Tyr(Me)$ AVP), i.v. (30 μg kg^{-1}), i.c. and i.c.v. (0.03 μg kg^{-1}) in mean arterial blood pressure (MAP), renal nerve activity (RNA) and heart rate (HR). Each point represents the mean value and the vertical lines show s.e.m. Changes caused by PSAB-OFB i.c. (*) were compared to saline, whereas changes caused PSAB-OFP pre-treated with V_1 antagonist (i.v.; i.c.; i.c.v.) were compared to antagonist control (*, data not shown for the sake of clarity) and changes caused by PSAB-OFP i.c. (†) compared to PSAB-OFP i.c.v. by using two-way ANOVA followed by the least significant difference test to compare the means. The bar below points indicates that those points have the same level of *P*. $^{*,\dagger}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$. ANOVA, analysis of variance.

With i.c. TC-2559 (3 μmol kg^{-1}, $n=5$; Figures 1d and 5a), as for PSAB-OFP i.c., the evoked rise in MAP had a faster onset, becoming significant after 2 min, reaching a maximum of 21 ± 7 mm Hg after 5 min. This was associated with, again, a delayed increase in RNA becoming significant after 5 min and reaching a similar maximum to that of PSAB-OFP i.c., of 86 ± 28% by 10 min. The rise in RNA caused by TC-2559 i.c. looks smaller than that observed via the i.c.v. route, but it is not significantly different. However, TC-2559 i.c. caused a now maintained significant tachycardia after 4 min of 17 ± 5 beats min^{-1} reaching a maximum of 24 ± 4 beats min^{-1} by 7 min (Figure 5a). Overall, TC-2559 administered i.c. compared with i.c.v. did not have any significant differences, except that the onset in pressor response was significantly faster when TC-2559 was administered i.c. (Figure 5a). This is similar to the observation for PSAB-OFP given i.c. compared with the i.c.v. route.

Baseline values for MAP and HR were 113 ± 14 mm Hg and 392 ± 59 beats min^{-1}.

Effects of the α4β2 antagonist dihydro-β-erythroidine and the α7 receptor antagonist methyllycaconitine i.c.v. on TC-2559 i.c.v.

In the presence of DhβE (10 μmol kg^{-1}; i.c.v.; $n=5$), TC-2559 (3 μmol kg^{-1}; i.c.v.) evoked a delayed rise in RNA, which was significantly attenuated. Furthermore, there was no significant associated rise in MAP and HR, apart from an increase in HR at 20 min (Figure 3b). In the presence of MLA (0.5 μmol kg^{-1}; i.c.v.; $n=5$), TC-2559 (3 μmol kg^{-1}; i.c.v.) had a very similar effect to TC-2559 alone, evoking the expected rise in MAP and RNA at 4 min of 31 ± 8 mm Hg and 67 ± 21%, reaching a maximum of 42 ± 11 mm Hg and 140 ± 15% after 9 min (Figure 3b). Interestingly, although there was a larger rise in blood pressure associated with a smaller increase in RNA, when compared to TC-2559 alone, these were not significantly different. There was

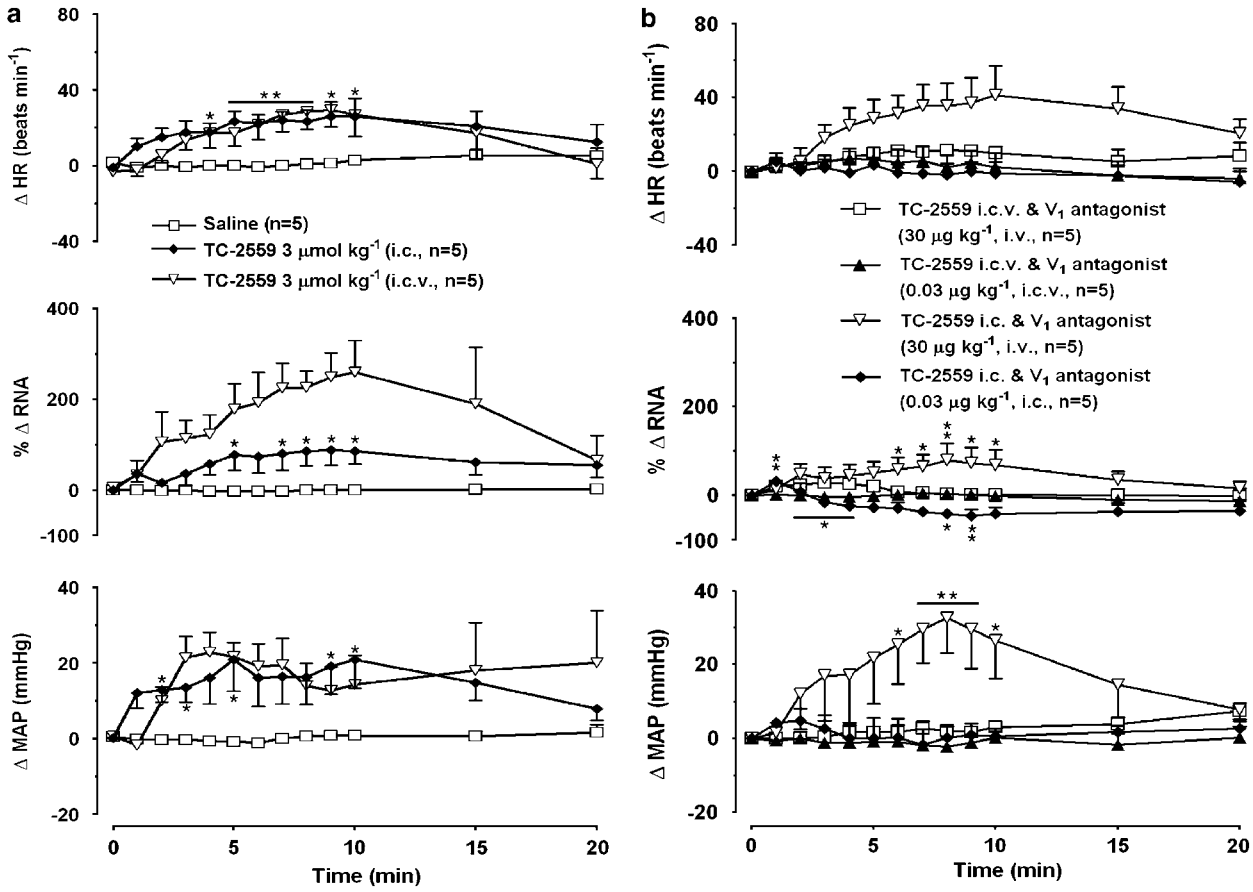

Figure 5 Anaesthetized artificially ventilated and neuromuscular blocked rats: a comparison of the changes (Δ) from baseline values over time (min) caused by TC-2559 (3 μmol kg^{-1}) (**a**) given i.c. and i.c.v. and saline (5 μl. i.c.) and (**b**) in the presence of V_1 receptor antagonist ($d(CH_2)_5Tyr(Me)$ AVP), i.v. (30 μg kg^{-1}), i.c. and i.c.v. (0.03 μg kg^{-1}) in mean arterial blood pressure (MAP), renal nerve activity (RNA) and heart rate (HR). Each point represents the mean value and the vertical lines show s.e.m. Changes caused by TC-2559 i.c. (*) were compared to saline, whereas changes caused by TC-2559 pre-treated with V_1 antagonist (i.v.; i.c.; i.c.v.) were compared to antagonist control (*, data not shown for the sake of clarity) by using two-way ANOVA followed by the least significant difference test to compare the means. The bar above or below points indicates that those points have the same level of *P*. *$P<0.05$ and **$P<0.01$. ANOVA, analysis of variance.

no significant change in HR for the duration of the experiment.

Baseline values for MAP and HR in the two groups were 113 ± 5 mm Hg and 361 ± 65 beats min^{-1} and 114 ± 14 mm Hg and 373 ± 34 beats min^{-1}, respectively.

Effects of the vasopressin V_1 receptor antagonist (i.v., i.c.v. and i.c.) on the effects of the α7 receptor agonist PSAB-OFP (i.c. and i.c.v.)

Pretreatment with the V_1 receptor antagonist for all routes (controls; $n=20$) failed to have any effect on the baseline variables.

In the presence of the V_1 receptor antagonist given i.v. (30 μg kg^{-1}), the effects of i.c.v. or i.c. PSAB-OFP (3 μmol kg^{-1}; $n=5$) on RNA and MAP were blocked (Figure 4b). However, for the i.c. route, there was now a small but significant fall in blood pressure of 13 ± 3 mm Hg after 2 min (Figure 4b) and a parallel nonsignificant decline in HR. Similarly, when the V_1 receptor antagonist (0.03 μg kg^{-1}) was given i.c.v., the evoked rises in RNA and MAP by i.c.v. PSAB-OFP were blocked and there was again a small and significant fall in MAP reaching 10 ± 2 mm Hg by 20 min (Figure 4b); however, the delay in onset was now 7 min. For the i.c. route, the V_1 receptor antagonist (0.03 μg kg^{-1}) against i.c. PSAB-OFP (3 μmol kg^{-1}, $n=5$) again blocked the expected pressor response and renal sympathoexcitation, although a significant transient increase in RNA (48 ± 18%) occurred at 1 min (Figure 4b). Again, a significant decrease in MAP was observed after 3 min, reaching a maximum of 11 ± 2 mm Hg by 5 min and returning to baseline by the end of the experiment (Figure 4b). This was paralleled by a nonsignificant decrease in HR. Overall, the V_1 receptor antagonist given by all routes of administration blocked the renal nerve excitation and pressor response evoked by PSAB-OFP given i.c.v. or i.c. In the case of central administration of the V_1 receptor antagonist, PSAB-OFP now evoked, by all central routes of administration, a decrease in blood pressure. The onset of this decrease in blood pressure was earlier for the i.c. than for the i.c.v. route. In this respect, i.c. PSAB-OFP also evoked a fall in blood pressure in the presence of i.v. V_1 receptor antagonist.

Baseline values for MAP and HR in the four groups were 113 ± 9 mm Hg and 401 ± 40 beats min^{-1}, 111 ± 8 mm Hg

and 367 ± 37 beats min^{-1}, 108 ± 15 and 107 ± 27 mm Hg and 397 ± 36 beats min^{-1} and 107 ± 27 mm Hg and 396 ± 43 beats min^{-1}, respectively.

The effect of pre-treatment with a vasopressin V_1 receptor antagonist (i.v., i.c.v. and. i.c.) on effects of the α4β2 receptor agonist TC-2559 (i.c. and i.c.v.)

In the presence of the V_1 receptor antagonist i.v. (30 μg kg^{-1}), the expected pressor and large, delayed, renal sympathoexcitation evoked by i.c.v. TC-2559 (3 μmol kg^{-1}; $n = 5$) were blocked. However, the tendency of RNA to increase between 2 and 4 min now became significant, reaching a maximum of 30 ± 6% after 3 min (Figure 5b). However, the expected effects evoked by i.c. TC-2559 (3 μmol kg^{-1}; $n = 5$) in the presence of the V_1 receptor antagonist i.v. (30 μg kg^{-1}) were overall similar in profile that is a rise in blood pressure, a delayed renal nerve excitation and tachycardia. The rise in MAP was now very variable and did not become significant until after 5 min (c.f. 2 min for TC-2559 alone), reaching a maximum of 33 ± 10 mm Hg at 8 min, which, although larger than that observed with TC-2559 i.c., was not significantly larger. The delayed increase in RNA was further delayed by 1 min not becoming significant until 6 min, reaching 80 ± 38% at 8 min. The expected tachycardia was unaffected (Figure 5b). The pressor and sympathoexcitation responses and delayed tachycardia evoked by i.c.v. TC-2559 (3 μmol kg^{-1}; $n = 5$) in the presence of V_1 receptor antagonist also given i.c.v. (0.03 μg kg^{-1}) were again blocked (Figure 5b). Similarly, the expected pressor responses, delayed sympathoexcitation and tachycardia evoked by i.c. TC-2559 (3 μmol kg^{-1}; $n = 5$) were also blocked by the V_1 receptor antagonist (0.03 μg kg^{-1}) given i.c. (Figure 5b). However, again there was an initial significant increase in RNA at 1 min of 32 ± 6%; this was followed by a significant decrease at 9 min reaching a maximum of 46 ± 15% at 10 min (Figure 5b).

Overall, the vasopressin antagonist pre-treatment blocked the expected effects of TC-2559 with exception of i.v. vasopressin on the evoked effects of TC-2559 i.c. Furthermore, i.c. TC-2559 in the presence of the V_1 receptor antagonist also given i.c. now evoked a delayed sympathoinhibition. Interestingly, TC-2559 i.c.v. and i.c. in the presence of the V_1 antagonist given i.v. and i.c., respectively, evoked an initial renal sympathoexcitation that was associated with no change in MAP or HR.

Baseline values for MAP and HR for the four groups were 108 ± 10 mm Hg and 378 ± 32 beats min^{-1}, 109 ± 6 mm Hg and 404 ± 41 beats min^{-1}, 109 ± 16 mm Hg and 387 ± 24 beats min^{-1}and 108 ± 13 mm Hg and 393 ± 35 beats min^{-1}, respectively.

Effects of vasopressin i.v. in the presence of the V_1 receptor antagonist given i.c.v. and i.c.

Cumulative doses (1, 3, 10, 30 ng kg^{-1}, i.v., $n = 7$) of vasopressin caused dose-related increases in MAP, reaching a maximum of 39 ± 7 mm Hg at 30 ng. In the presence of the V_1 receptor antagonist given i.c.v. or i.c. (0.03 μg kg^{-1}; $n = 7$), the effects of i.v. vasopressin were unaffected (data not shown).

Effects of i.v. infusions of PSAB-OFP and TC-2559

Infusion i.v. of PSAB-OFP or TC-2559 (3 μmol kg^{-1}, $n = 3$) over 3 min caused no significant effect on MAP, RNA or HR when compared to saline infusion ($n = 3$) control. Baseline values for MAP and HR were 128 ± 6 and 116 ± 5 mm Hg and 403 ± 30 and 423 ± 14 beats min^{-1}, respectively.

Discussion

The ability of central administration of the α7 nAChR agonist PSAB-OFP to cause a dose-related increase in RNA and blood pressure, and the observation that these effects are blocked by pre-treatment with the α7 nAChR antagonist MLA (Ward *et al*., 1990), but not by pre-treatment with an effective dose (see below) of the α4β2 nAChR antagonist (DhβE; Bencherif *et al*., 2000), indicates that these effects are due to activation of α7 nAChRs. Similarly, the α4β2 nAChR agonist TC-2559 given centrally caused rises in RNA and blood pressure, which again were blocked by the α4β2 nAChR antagonist DhβE, but not by MLA, the latter given at dose known to block α7 nAChRs. This confirms that the effects of TC-2559, at least at the middle dose, are mediated by the α4β2 nAChRs.

The high dose of PSAB-OFP also caused a tachycardia, which may or may not be mediated by α7 nAChRs, whereas the high dose of TC-2559 caused renal sympathoinhibition associated with a tendency for blood pressure to fall suggesting, as these effects are in the opposite direction, that they are not mediated by α4β2 nAChRs. In this respect, *in vitro* studies have shown that TC-2559 is selective for α4β2 receptors with an EC_{50} of 0.18 μM. However, at high concentrations, it also has affinity for α2β4, α3β4 and α4β4 nAChRs, with an $EC_{50} = 10$–30 μM for these other β4 containing receptors (Chen *et al*., 2003). Therefore, the largest dose of TC-2559 could be activating these additional nAChRs to cause a decrease in blood pressure and sympathoinhibition. Furthermore, although both agonists were given centrally, there is a delay in their effects being observed, especially when administered by i.c.v. injection. This could be interpreted as being, because these agonists have leaked out of the brain into the periphery; however, as no effects were observed with peripheral administration of these agonists, this seems highly doubtful.

Thus, it can be concluded that activation of either central α7 or α4β2 nAChRs evokes renal sympathoexcitation and a rise in blood pressure. Furthermore, although the effects of activation of both these receptor subtypes were very similar, the present evidence indicates that these receptors act independently to evoke these actions as the antagonist studies did not indicate any crossover, that is the α7 nAChR antagonist failed to block the effects of α4β2 receptor agonist. In addition, the data suggest that activation of additional nAChR subtypes can have the opposite effects to those of α7 and α4β2 nAChRs in this system.

Vasopressin release

In the present experiments, pretreatment with an i.v. V_1 receptor antagonist blocked the expected increases in MAP and RNA induced by PSAB-OFP and TC-2559 given i.c.v., as well as the delayed tachycardia caused by TC-2559. This indicates that all the effects of these selective agonists, via the i.c.v. route, are mediated by the release of vasopressin presumably into the circulation. This would also apply to i.c. PSAB-OFP.

TC-2559 i.c. in the presence of the V_1 vasopressin receptor antagonist i.v. had a similar profile of effects to TC-2559 alone, although initially the pressor response was very variable, only reaching significance after 8 min. Similarly, the increase in RNA evoked by TC-2559 i.c. was delayed in the presence of the V_1 receptor antagonist (i.v.). These latter observations are not consistent with the view that the effects of i.c. TC-2559 are mediated by the release of vasopressin into the circulation. However, it is possible that the V_1 receptor antagonist given i.v. could have entered the brain to interfere with the effects of these agonists. Thus, a series of experiments were carried out to determine whether the central block of V_1 receptors was responsible for some of the effects observed. The dose of V_1 vasopressin antagonist that was chosen to be given centrally was shown not to leak into the periphery as determined by its failure to block the evoked pressor response to i.v. vasopressin (see results section). In the presence of this dose of the V_1 vasopressin antagonist given centrally (i.c.v. or i.c.), the pressor and renal sympathoexcitatory effects of PSAB-OFP and TC-2559 were nearly completely blocked, implying that these effects are mediated by the central release of vasopressin. Thus, a possible reason for the failure of i.v. vasopressin antagonist to clearly interfere with the responses evoked by i.c. TC-2559 may simply be due to the concentration that is reached in the brainstem in that it is at the lower end of the range to cause the degree of block required. This would be consistent with the large variability of the effect of the i.c. TC-2559 at least on blood pressure. Overall, the present data indicate that the evoked excitatory cardiovascular effect of activation of either α7 or α4β2 nAChRs is mediated primarily by the central release of vasopressin.

Site of action

The renal sympathoexcitation and pressor response evoked by PSAB-OFP and TC-2559 i.c.v. were not rapid in onset and instead required at least 4 min for significant increases to be observed, whereas when these agonists were given i.c., the rise in MAP was significantly more rapid, although the onset of renal sympathoexcitation was still delayed. This would suggest that the major site of action for these agonists, to at least cause the pressor response, is at the level of the brainstem. This is in agreement with previously published data that demonstrated that nicotine was more effective in inducing vasopressin release into the circulation, when applied to the hindbrain rather than the forebrain (Bisset *et al*., 1975; Castro de Souza and Rocha E Silva, 1977).

The three major cardiovascular areas in the brainstem are the nucleus tractus solitarius (NTS), the site of cardiovascular afferent termination (see Jordan and Spyer, 1986), the rostral ventrolateral medulla (RVLM), the site of pre-vasomotor sympathetic neurones (see Dampney, 1994), and the caudal ventrolateral medulla (CVLM) the site of tonic inhibitory drive to the RVLM and part of the baroreflex pathway (see Schreihofer and Guyenet, 2002). The NTS also sends projections to the RVLM and the CVLM (see Dampney, 1994, Schreihofer and Guyenet, 2002), and the neurosecretory cells in the paraventricular nucleus and the evidence indicates that this latter pathway is noradrenergic (Sawchenko and Swanson, 1982; Dampney, 1994; Valentine *et al*., 1996; Hermes *et al*., 2006). Furthermore, application of nicotine to the NTS not only causes noradrenaline to be released at the level of the paraventricular nucleus (Zhao *et al*., 2007), but also causes a decrease in blood pressure and HR (Tseng *et al*., 1993). This action of nicotine may (Dhar *et al*., 2000) or may not (Ferreira *et al*., 2002) be mediated in part by α7 receptors, although α7 nAChRs and α4β2 nAChRs have been shown to be located in the NTS (Dominguez del *et al*., 1994; Ferreira *et al*., 2002). However, injections of vasopressin into the NTS cause a rise in blood pressure and increase plasma noradrenaline implying that the sympathetic has been activated (King and Pang, 1987); however, the HR is unaffected. In this respect, the paraventricular nucleus is known to send a vasopressin-containing projection to the NTS (Sawchenko and Swanson, 1982; Dampney, 1994). Thus, the above observations support the view that these agonists could be acting at the level of the NTS, although how the release of vasopressin is evoked at the level of the NTS is unclear. For instance, does the activation of these nicotinic receptors in the NTS cause the release of vasopressin by acting directly on vasopressin-containing fibres or neurones within the NTS and/or by activating an ascending pathway to the paraventricular nucleus, which then activates a descending vasopressin-containing pathway causing the local release of vasopressin in the NTS (indirectly)?.

Another site of action could be the RVLM, and electrical stimulation of this area is known to cause a doubling of the level of plasma vasopressin (Ross *et al*., 1984), and vasopressin applied or injected into the RVLM causes a rise in blood pressure; however, HR effects were very variable. These effects are mediated by V_1 receptors (Andreatta-Van Leyen *et al*., 1990; Gomez *et al*., 1993). Similar effects are also observed when nicotine is injected into the RVLM (Tseng *et al*., 1993), and this area is also known to contain α4β2 and α7 nAChRs (Wada *et al*., 1989). In this respect, there is evidence that the C1 (adrenaline-containing) neurons, which are located in the caudal part of the RVLM do project to the paraventricular nucleus thus activation of these neurones in the RVLM could cause vasopressin release (Cunningham *et al*., 1990). In addition, evidence also indicates that there is a vasopressin-containing pathway from the paraventricular nucleus to the RVLM (Yang and Coote, 1998; Yang *et al*., 2001). Similarly, as for the NTS, it is not clear whether these agonists are causing direct release of vasopressin into this area or indirectly by activation of an ascending and then a descending pathway from the paraventricular nucleus.

As for the CVLM, a projection may exist from the paraventricular nucleus to the CVLM, as the CVLM overlaps

with the A1 area, which projects to the paraventricular nucleus and also forms part of the NTS. However, how functionally distinct the CVLM GABAergic interneurons are from the A1 region is open to conjecture (see Dampney, 1994). In fact, in the first description of the CVLM, application of nicotine to this area caused vasopressin release and a fall in blood pressure (Bisset *et al*., 1975; Feldberg and Guertzenstein, 1976). This is believed to be in part mediated by α7 nAChRs (Aberger *et al*., 2001). Thus, it is also possible that the release of vasopressin into the circulation is due to an action at the level of the CVLM or the A1 area; however, falls in blood pressure would be predicted, although this may be counteracted by sympathoexcitatory actions from these agonists in other areas. In this respect, it is interesting to note that PSAB-OFP causes falls in blood pressure when central V_1 receptors are blocked. Furthermore, the experiments do not completely rule out an action of these agonists at the level of the paraventricular nucleus, although the observed delay in onset would indicate that such an action is minor. Thus, at present, it is difficult to rule out any of these major brainstem cardiovascular areas as the site/s of action of these agonists.

Mechanisms involved in the pressor response

The rise in blood pressure evoked by the two agonists, as indicated above, can involve the release of vasopressin into the periphery along with central sympathoexcitation, both of which would cause vasoconstriction and, in the case of increased sympathetic outflow, an increase in cardiac output, although HR data from the present experiments suggest that this is unlikely. If blood pressure, renal sympathetic activity and HR all increased rapidly and in parallel, this would be consistent with the agonist causing generalized sympathoexcitation. If vasopressin was released alone into the circulation, the evoked increase in blood pressure would be expected to activate baroreceptors to cause a reflex bradycardia and central inhibition as seen for central 5-HT-pathway-mediated vasopressin release (Anderson *et al*., 1992, 1996). However, the effects of these nAChR agonists at the level of the NTS, the site of termination of baroreceptor afferents, could easily interfere with such a feedback system and, further, central sympathoexcitatory activity could override such a system. The evidence for additional sympathoexcitatory actions of these agonists to resistance vessels in the present experiments has not been proved, nor the fact that vasopressin is released into the circulation, because the i.v. V_1 receptor antagonist may have entered the CNS and caused central as well as peripheral block of the effects of vasopressin. However, the published data from the central application of nicotine (see above) indicate that these agonists would be expected to cause the release of vasopressin into the circulation. Furthermore, the present data that TC-2559 i.c. causes a rise in blood pressure in the presence of the V_1 receptor antagonist administered i.v. would support this suggestion that sympathoexcitation to resistance vessels is occurring.

Data from the present experiments also indicate that, at least for the renal sympathetic outflow, the onset of excitation is delayed whether these agonists are administered i.c.v. or i.c., suggesting that the increase in blood pressure is independent of renal sympathoexcitation. Thus, renal sympathoexcitation could be secondary to the rise in blood pressure. It is therefore possible that renal sympathoexcitation could be due to circulating vasopressin feedback probably at the level of the area postrema, which has a direct innervation to the NTS (Shapiro and Miselis, 1985). However, data from rabbits indicate that such feedback causes renal sympathoinhibition (see Nishida and Bishop, 1992). Another possibility is that the delay could be due to these agonists activating ascending pathways to the paraventricular nucleus, which then activates a vasopressin-releasing projection to the RVLM, causing sympathoexcitation. Thus, the mechanism for this delay remains to be determined although the sympathoexcitation also involves the release of vasopressin at the level of the brainstem.

Conclusion

The present data indicate that the pressor actions due to activation of central α7 or α4β2 nAChRs is primarily due to the release of vasopressin at the level of the brainstem involving mainly the NTS and RVLM. The rise in blood pressure is attributed also to the release of vasopressin into the circulation along with sympathoexcitation to resistance beds. This was associated surprisingly with little effect of the agonists on HR. Furthermore, it is surprising that both agonists evoked a similar profile of cardiovascular effects, and that these receptors seem to be acting independently of each other. As neither selective antagonist had any effect on baseline variables, this would also indicate that these receptors are not under tonic activation, at least in the present experimental conditions. Thus, the primary action of α7 or α4β2 nAChRs in central cardiovascular regulation is to release vasopressin centrally and peripherally.

Acknowledgements

CM was supported by a BBSRC collaborative studentship with Eli Lilly UK. We thank Mr S Wilkinson for valuable technical assistance.

Conflict of interest

The authors state no conflict of interest.

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