

**Donitriptan selectively decreases jugular venous oxygen  
saturation in the anesthetized pig: Further insights  
into its mechanism of action relevant to headache relief**

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## Abstract

The effects of donitriptan on systemic arterial-jugular venous oxygen saturation difference were evaluated in pentobarbitone-anesthetized pigs. Oxygen and carbon dioxide partial pressures in systemic arterial and jugular venous blood as well as hemoglobin oxygen saturation were determined by conventional blood gas analysis. Vehicle (40 % PEG in saline, n=9) or donitriptan (0.01, 0.04, 0.16, 0.63, 2.5, 10 and 40  $\mu\text{g}/\text{kg}$ , n=7) were cumulatively infused over 15 min. per dose. The involvement of 5-HT<sub>1B</sub> receptors was assessed in the presence of the 5-HT<sub>1B/1D</sub> receptor antagonist, GR 127935. Donitriptan decreased markedly and dose-dependently jugular venous oxygen saturation [ED<sub>50</sub> 0.5 (0.3-1.1)  $\mu\text{g}/\text{kg}$ ], in parallel with increases in carotid vascular resistance [ED<sub>50</sub> 0.9 (0.7-1.1)  $\mu\text{g}/\text{kg}$ ]. Since arterial oxygen saturation and partial pressure remained unchanged, donitriptan significantly increased arteriovenous oxygen saturation difference from 0.63  $\mu\text{g}/\text{kg}$  (max. variation:  $57 \pm 18$  %,  $P < 0.05$  compared to vehicle). Unexpectedly, donitriptan from 2.5  $\mu\text{g}/\text{kg}$  induced marked and significant increases in carbon dioxide partial pressure (PvCO<sub>2</sub>) in venous blood (max. increase  $18.8 \pm 5.7$  %;  $P < 0.05$  compared to vehicle). Pretreatment with GR 127935 (0.63 mg/kg, n=5) abolished the fall in venous oxygen saturation, the increase in carotid vascular resistance and reduced the increases in PvCO<sub>2</sub> induced by donitriptan.

The results demonstrate that donitriptan, via 5-HT<sub>1B</sub> receptor activation, decreases the oxygen saturation of venous blood draining the head, concomitantly with cranial vasoconstriction. Since donitriptan also increased PvCO<sub>2</sub>, an effect upon cerebral oxygen consumption and metabolism is suggested in addition to cranial vasoconstriction which may be relevant to its headache relieving effects.

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Donitriptan is a unique high-efficacy agonist at 5-HT<sub>1B/1D</sub> receptors which is currently being evaluated for efficacy in the acute relief of migraine headache in phase II clinical trials (Dukat 2001; John *et al.*, 1999; 2000). One of the key pharmacological actions of donitriptan is 5-HT<sub>1B</sub> receptor-mediated cranioselective vasoconstriction (Tom *et al.* 2002; Van den Broek *et al.* 2002a), due chiefly to closure of cephalic arteriovenous anastomoses (AVAs; Tom *et al.*, 2002). Immunohistochemical studies in the human trigemino-cerebrovascular system have demonstrated that 5-HT<sub>1B</sub> receptors are localized to blood vessels, but not 5-HT<sub>1D</sub> receptors (Longmore *et al.*, 1997). Closure of cephalic AVAs is a common feature of tryptamine-derived, selective 5-HT<sub>1B/1D</sub> receptor agonists (triptans; De Vries *et al.*, 1999a) which may be associated with increases in systemic arterial-jugular venous oxygen saturation difference (AVOSD; De Vries *et al.*, 1996; Tom *et al.*, 2002). Before the advent of the triptans, the non-selective 5-HT<sub>1B</sub> receptor agonist, ergotamine (Villalon *et al.*, 1999) was also shown to elicit selective carotid vasoconstriction confined to cephalic AVAs (Johnston & Saxena, 1978). Moreover, increases in AVOSD due to decreases in jugular venous oxygen saturation and oxygen partial pressure (PO<sub>2</sub>) without affecting systemic arterial oxygen saturation or PO<sub>2</sub> were also observed in this study (Johnston & Saxena, 1978). However, it is presently unknown whether the recently described triptan 5-HT<sub>1B/1D</sub> receptor agonist-induced increases in AVOSD are due to changes in systemic arterial or jugular venous oxygen saturation, or both.

The aim of the present investigation was therefore to elucidate the mechanism of donitriptan-induced increases in AVOSD. The results indicate that donitriptan selectively decreases jugular venous PO<sub>2</sub> and hemoglobin oxygen saturation whilst increasing jugular venous PCO<sub>2</sub> concomitantly with carotid vasoconstriction. The results are discussed in the light of established mechanisms of action of triptan 5-HT<sub>1B/1D</sub> receptor agonists in relation to migraine relief.

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## Methods

### *Animal preparation*

Twenty-seven male Landrace pigs (18-24 kg, M. Gaec, Sorèze, France) were premedicated with intramuscular administration of azaperone (3 mg/kg) after an overnight fast. General anesthesia was then induced with sodium pentobarbitone (25 mg/kg, i.v., Sanofi, France). The animals were intubated, ventilated by positive pressure (Alpha 100, Minerve, Esternay, France) and anesthesia was maintained with a continuous infusion *via* the right saphenous vein of sodium pentobarbitone (6-18 mg/kg/h). Respiratory rate and tidal volume were carefully adjusted for maintaining blood gases within physiological limits (ABL 510, Radiometer, Copenhagen, Denmark). Body temperature was maintained constant between 37 and 38.5 °C by a servo-controlled heating blanket.

A standard four-limb ECG in lead II was monitored throughout the experiments. Fluid-filled catheters were inserted into the descending thoracic aorta *via* the right femoral artery and left saphenous vein for continuously monitoring arterial pressure and drug administration, respectively. Blood was withdrawn through this arterial line, and the right external jugular vein was catheterized for venous blood sampling in order to obtain measurements of arterial and venous blood gases. Another catheter was introduced into the left ventricle *via* the left femoral artery to determine left ventricular pressure (LVP). Subsequently, left and right common carotid arteries were carefully cleaned of surrounding connective tissue, then blood flows in each artery were measured with pulsed Doppler flow probes (Crystal Biotech, Northborough, USA). The mean total carotid blood flow (TCBF) was calculated as the sum of left and right mean carotid blood flows.

A left lateral thoracotomy was performed in the fourth intercostal space and the pericardium was opened. Aortic blood flow (ABF) was measured with an electromagnetic flow probe (SP

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2202, Gould, USA) placed around the thoracic aorta. The left anterior descending coronary artery (LAD) was isolated and a pulsed Doppler flow probe was placed at its proximal level.

Systemic and regional vascular resistances were determined as the ratio of mean arterial pressure to aortic blood flow or mean Doppler flows, except for LAD vascular resistance (LADVR) calculated by dividing coronary perfusion pressure (i.e., the difference between diastolic arterial pressure and left ventricular end-diastolic pressure) by LAD blood flow.

These parameters are the average of all successive determinations taken during a 30 s recording period. Analog phasic pressure and flow outputs were fed simultaneously to an amplifier-recorder (Gould) and to a personal computer equipped with an analog-to-digital converter board. Using Data flow® software (Crystal Biotech, Northborough, MA), analog signals were digitized every 5 msec for high resolution, and subsequently stored on optical disk.

An acceptable estimate of blood flow (Q) was calculated by the following equation:

$$Q = 1.25 d^2 \Delta f$$

where d is the vessel's inside diameter in millimeters and  $\Delta f$  is the Doppler shift in kHz.

Several blood gas parameters were determined: The oxygen and carbon dioxide partial pressure values in arterial and venous blood,  $PaO_2$ ,  $Paco_2$ ,  $PvO_2$  and  $PvCO_2$  respectively, arterial and venous pH. The arterial and venous oxygen saturation was calculated by the following equation:

$$OS = c_{O_2 Hb} \times 100 / c_{O_2 Hb} + c_{RHb}$$

where OS is the arterial or venous oxygen saturation,  $c_{O_2 Hb}$  is the concentration of oxyhemoglobin and  $c_{RHb}$  is the concentration of reduced hemoglobin.

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The arteriovenous oxygen saturation difference (AVOSD) was calculated as AOS minus the jugular venous oxygen saturation (VOS). This difference is an index of cranial O<sub>2</sub> extraction.

### *Experimental protocol*

Experiments were carried out in two separate groups of pigs: vehicle (a mixture of 40 % polyethyleneglycol 300 in sterile saline, 0.9 %, n=9), and donitriptan (n=7). After completion of surgical procedures, a stabilization period of at least 30 min was observed.

Donitriptan was infused in cumulative fashion over the following incremental dose range: 0.01; 0.04; 0.16; 0.63; 2.5; 10 and 40 µg/kg. Each dose of drug was infused over 15 min. Preliminary studies showed that 15 min per dose was more than sufficient to achieve steady-state responses. Similarly, seven 15 min. cumulative administrations of vehicle were infused in the vehicle group.

An additional group was constituted to study the effects of donitriptan (n=5) in presence of GR 127935, a relatively selective 5-HT<sub>1B/1D</sub> receptor antagonist (Skingle *et al.*, 1996). The effects of GR 127935 alone were evaluated in a further group (n=6). In all experiments GR 127935 was infused over 2 h at 0.63 mg/kg (De Vries *et al.*, 1996) starting 15 min before donitriptan administration.

### *Drugs*

Azaperone was purchased from Janssen Pharmaceutica (Belgium). Donitriptan: 4-[4-[2-[3-(2-amino-ethyl)-1H-indol-5-yloxy]-acetyl]-piperazin-1-yl]-benzotrile hydrochloride and GR 127935: *N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2[-methyl-4(5-methyl-1,2,4)-oxadiazol-3-yl]-(1,1 biphenyl)-4-carboxamide dihydrochloride, were synthesized by the Department of Analytical Chemistry and Division of Medicinal Chemistry IV at the Centre

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de Recherche Pierre Fabre (Perez and John, 1999). Donitriptan was dissolved in 40 % polyethyleneglycol 300 in sterile saline (0.9 %) whereas GR 127935 was dissolved in sterile saline (0.9 %). Drugs were weighed as base taking into account the salt to base ratio.

#### *Data and statistical analysis*

Dose-response curves were fitted using an operational sigmoid model (Origin, Microcal Software, Northampton, Mass., USA) from relative maximal effects induced by agonists. From the results of these analyses, the geometric mean dose of agonist producing 50 % of the maximal response ( $ED_{50}$ ) was calculated with 95 % confidence intervals.

Parameters were measured at the end of baseline period and the end of each 15 min infusion period. All values were expressed as means  $\pm$  SEM. One-factor analysis of variance (ANOVA) with repeated measurements followed by Dunnett's test (Sigma Stat, Jandel, Erkrath, Germany) was used to assess significance among and between groups, and the unpaired Student's t test as an additional post-hoc test was employed when appropriate (Sigma Stat).

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## Results

### *Effects of donitriptan on blood gas parameters*

The effects of donitriptan on blood gases are presented in Table 1. The initial oxygen partial pressure values in arterial blood ( $\text{PaO}_2$ ) were comparable among both groups ( $P=\text{NS}$ ). In vehicle-treated animals  $\text{PaO}_2$  slightly but significantly decreased throughout the experiment reaching  $114.2 \pm 6.3$  mmHg by the end of experiments from an initial value of  $122.5 \pm 5.7$  mmHg ( $P<0.05$ , Table 1). In donitriptan-treated animals a comparable slight decrease in  $\text{PaO}_2$  occurred. The oxygen partial pressure in jugular venous blood ( $\text{PvO}_2$ ) was also determined in both groups. In the vehicle group,  $\text{PvO}_2$  remained unchanged throughout the experiments ( $P=\text{NS}$ , Table 1). Donitriptan moderately but significantly decreased  $\text{PvO}_2$  only at  $40 \mu\text{g}/\text{kg}$  compared to vehicle, the maximal decrease reached  $31 \pm 6\%$  ( $P<0.05$ ). The initial values of  $\text{PaCO}_2$  measured in the donitriptan-treated group were similar to those of the vehicle group. In both groups,  $\text{PaCO}_2$  values did not significantly change between the beginning and the end of the experiments. Baseline  $\text{PvCO}_2$  values did not significantly differ between vehicle and donitriptan groups. Vehicle was devoid of significant effects *per se* on  $\text{PvCO}_2$  as indicated in Table 1. On the other hand, donitriptan from  $2.5 \mu\text{g}/\text{kg}$  induced marked and significant increases in  $\text{PvCO}_2$  ( $P<0.05$  compared to vehicle group, Table 1). This constitutes one of the major findings of the present study. It is interesting to note that no significant variations in pH were found in the arterial or venous blood analyses (Table 1).

Baseline AOS values were similar in the different treatment groups ( $P=0.09$ ). AOS was not significantly affected by vehicle or donitriptan ( $P=\text{NS}$ ). In the vehicle group, baseline and end-experimental AOS values were  $99.0 \pm 0.4$  and  $98.6 \pm 0.6\%$ , respectively. The same time points in donitriptan-treated animals were:  $100.1 \pm 0.1$  and  $96.9 \pm 2.1\%$  ( $P=\text{NS}$ , Table 1). These results are illustrated in Fig 1 which represents the percent changes from baseline

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values for AOS and VOS. In the vehicle group, VOS remained unchanged throughout the experiment ( $68.8 \pm 3.5$  and  $68.0 \pm 3.7$  %, initial and end-experiment values, respectively,  $P=NS$ , Table 1). In donitriptan-treated animals, VOS decreased dose-dependently (geometric mean  $ED_{50}$  value :  $0.45 \mu\text{g/kg}$  and 95 % confidence limits  $0.27 - 1.06 \mu\text{g/kg}$ ) leading to statistically significant differences *versus* vehicle group that occurred from  $0.63$  to  $40 \mu\text{g/kg}$  (Fig 1). VOS decreased by  $32 \pm 8$  and  $30 \pm 9$  % at donitriptan  $10$  and  $40 \mu\text{g/kg}$ , respectively (both  $P<0.05$  compared to vehicle group). AOS remained unchanged throughout the experiment. Consequently, decreases in  $PvO_2$  determined the increases in AVOSD. Indeed, AVOSD significantly increased from  $0.63 \mu\text{g/kg}$  (maximal variation:  $57 \pm 18$  %,  $P<0.05$  at  $10 \mu\text{g/kg}$  compared to vehicle group, Fig 2 A). In vehicle-treated animals, AVOSD did not undergo significant changes ( $P=NS$ , Table 1).

#### *Effects of donitriptan on hemodynamic parameters*

The effects of donitriptan on blood pressure, heart rate (HR) and blood flows are summarized in Table 2. Under baseline conditions, these cardiovascular parameters were not significantly different from those in the vehicle group, with the exception of TCBF which was significantly higher in donitriptan than in controls, ( $P<0.05$ , Table 2).

In vehicle-treated animals, MAP and LVP did not undergo notable changes throughout the experiments. As indicated in Table 2, HR gradually decreased with time in this group. Aortic and coronary blood flows tended to decrease with time whereas TCBF remained constant throughout the experiments. Vascular resistances (SVR, TCVR and LADVR) were not significantly affected by vehicle when compared to initial values, maximal changes in SVR, TCVR and LADVR reached:  $14 \pm 10$ ,  $P=NS$ ;  $-3 \pm 6$ ,  $P=NS$  and  $18 \pm 10$  %,  $P=NS$ , respectively.

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Donitriptan elicited no or weak increases in MAP and LVP (Table 2). Similar small decreases in HR were obtained with donitriptan or vehicle. Donitriptan markedly decreased TCBF (maximal variation  $-50 \pm 3$  %,  $P < 0.05$ , *versus* vehicle) whereas ABF and LADBF were not significantly affected compared to vehicle (Table 2). The vascular selectivity of donitriptan was assessed by the measurement of relative vascular resistances. Donitriptan evoked dose-dependent increases in TCVR leading to a  $132 \pm 10$  % maximal increase (at  $40 \mu\text{g/kg}$ ,  $P < 0.05$  compared to vehicle, Fig 2 B) with a geometric mean  $\text{ED}_{50}$  value of  $0.9 \mu\text{g/kg}$  (95 % confidence limits:  $0.72$ - $1.12 \mu\text{g/kg}$ ). It is interesting to note that donitriptan increased TCVR with a geometric mean  $\text{ED}_{50}$  value of  $0.9 \mu\text{g/kg}$  being not statistically significantly different to that calculated for decreasing VOS ( $0.45 \mu\text{g/kg}$ ,  $P = 0.195$ ). These increases in TCVR were significantly greater than corresponding changes in SVR and LADVR. Donitriptan produced no significant changes in LADVR but significantly increased SVR from  $0.16 \mu\text{g/kg}$  (maximal increase  $58.3 \pm 14.8$  % at  $10 \mu\text{g/kg}$ ,  $P < 0.05$  *versus* vehicle group).

#### *Effects of GR 127935*

In an additional series of experiments, donitriptan was re-examined in animals pretreated by GR 127935 ( $0.63 \text{ mg/kg}$ ), a relatively selective  $5\text{-HT}_{1\text{B}/1\text{D}}$  receptor antagonist (Skingle *et al.*, 1996). Under these conditions, baseline  $\text{PaO}_2$ ,  $\text{PvO}_2$ ,  $\text{PaCO}_2$ ,  $\text{PvCO}_2$ , AOS, VOS and AVOSD values were similar in vehicle- and donitriptan-treated animals (data not shown). GR 127935 alone did not significantly modify these parameters. The maximal changes in AVOSD induced by GR 127935 alone were  $22 \pm 11$  % ( $P = 0.27$  *versus* vehicle without GR 127935). In the presence of GR 127935, in vehicle- or donitriptan-treated animals a comparable, slight decrease in  $\text{PaO}_2$  occurred (maximal variation,  $-7.1 \pm 4.8$  and  $-5.1 \pm 2.4$  mmHg,  $P = 0.68$ , respectively). In the same manner,  $\text{PvO}_2$  was moderately decreased but not significantly and this tendency appeared in both groups. GR 127935 reduced the donitriptan-induced increase

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in  $PvCO_2$  ( $P=NS$  versus vehicle, data not shown). AOS remained constant during the experiments in vehicle- and donitriptan-treated animals, maximal variations were  $-0.3 \pm 0.3$  and  $-0.5 \pm 0.5$  %, respectively. The effects of GR 127935 on donitriptan-induced decreases in VOS were also studied. GR 127935 abolished the fall in VOS induced by donitriptan. The maximal changes in vehicle- and donitriptan-treated pigs were  $-19 \pm 10$  and  $-9 \pm 3$  %, respectively ( $P=0.38$ ). Thus, in the presence of GR 127935, donitriptan-induced increases in AVOSD were abolished (maximal variation  $29 \pm 19$  % versus  $22 \pm 11$  % in vehicle group, Fig 2 A).

GR 127935 *per se* induced a significant increase in TCVR (maximal change:  $24 \pm 4$  %,  $P<0.05$  compared to vehicle, Fig 2 B). On the other hand, no significant effects of GR 127935 on the other parameters were noted (except for TCBF, maximal change  $-17 \pm 3$  %,  $P<0.05$  compared to vehicle). The effects of donitriptan on TCBF were antagonized by GR 127935. Under these conditions, the maximal decrease in TCBF in the donitriptan group reached  $-20 \pm 7$  %, ( $P=0.60$  compared to vehicle). Figure 2 B shows that GR 127935 markedly antagonized the variations in TCVR induced by donitriptan  $40 \mu\text{g}/\text{kg}$ , maximal change:  $-24 \pm 9$  % ( $P=0.79$  compared to vehicle).

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## Discussion

The mechanism of donitriptan-induced increases in arteriovenous oxygen saturation difference (AVOSD) was investigated in the anesthetized farm pig. Donitriptan dose-dependently increased AVOSD and decreased jugular venous hemoglobin oxygen saturation from 0.63  $\mu\text{g/kg}$  i.v. without affecting systemic arterial oxygen saturation. Donitriptan-induced increases in AVOSD are therefore mediated by decreased jugular venous oxygen saturation. These effects were accompanied by decreases in jugular venous  $\text{PO}_2$  and increases in  $\text{PCO}_2$ , demonstrating augmented cephalic oxygen consumption and metabolism. Donitriptan also concomitantly and dose-dependently reduced carotid blood flow from 0.16  $\mu\text{g/kg}$ . The 5-HT<sub>1B/1D</sub> receptor antagonist, GR 127935 (Skingle *et al.*, 1996) significantly reduced both donitriptan-induced increases in AVOSD and carotid vascular resistance, indicating the likely involvement of 5-HT<sub>1B</sub> receptors. The present results are in agreement with a previous study (John *et al.*, 1999) and those of a recent study (Tom *et al.*, 2002), which showed dose-dependent, 5-HT<sub>1B</sub> receptor-mediated decreases in carotid vascular conductance due to a highly selective reduction in cephalic arteriovenous anastomotic (AVA) blood flow. These latter effects were accompanied by an increase in AVOSD (Tom *et al.*, 2002). Indeed, since constriction of cephalic AVAs is a model which has a predictive value for antimigraine activity (Tfelt-Hansen *et al.*, 2000), donitriptan should be effective in the acute treatment of migraine.

### *Donitriptan-induced increases in AVOSD*

Donitriptan dose-dependently reduced jugular VOS and  $\text{PvO}_2$  without significantly affecting systemic AOS or  $\text{PaO}_2$ . Consequently the donitriptan-induced increases in AVOSD can be explained by the selective decreases in jugular VOS. The most plausible explanation for the

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selective decreases in jugular VOS and PVO<sub>2</sub> evoked by donitriptan is closure of cephalic AVAs which underlies 5-HT<sub>1B</sub> receptor-mediated cranial vasoconstriction in the present model (Tom *et al.*, 2002). An unexpected finding in the present study, however, was that donitriptan also dose-dependently and significantly increased jugular PvCO<sub>2</sub>. Consequently, an additional effect of donitriptan in enhancing cerebral metabolism must also be considered. Augmented PvCO<sub>2</sub> concomitant with decreases in PVO<sub>2</sub> are hallmarks of increased tissue metabolism (Dejours, 1963). Systemic AOS or PO<sub>2</sub> were not modified by donitriptan, thereby allowing effects on hemoglobin affinity for oxygen or on pulmonary blood oxygenation to be ruled out. Metabolic acidosis can also be excluded, because no variations in arterial or venous pH or PaCO<sub>2</sub> were detected. Donitriptan significantly decreased jugular venous PO<sub>2</sub> and hemoglobin oxygen saturation, indicating enhanced cephalic oxygen extraction. However one of the main observations of the present study is the marked and dose-dependent increase in jugular venous PCO<sub>2</sub> in the absence of changes in arterial PCO<sub>2</sub> which were consistently and robustly elicited by donitriptan. This strongly suggests that in addition to promoting cephalic oxygen extraction from arterial blood, as indicated by significant decreases in venous oxygen saturation and PO<sub>2</sub>, donitriptan also enhances tissue metabolism, as indicated by significant increases in jugular venous PCO<sub>2</sub>. It is noteworthy that physiological arteriovenous differences in PCO<sub>2</sub> are small (around 5-7 mmHg; compared to a 50-60 mmHg difference for PO<sub>2</sub>), indicating a relatively low sensitivity of this parameter to changes in tissue metabolism. This is due to larger (around 17 fold) tissue reserves of dissolved CO<sub>2</sub> compared to O<sub>2</sub> (Farhi and Rahn, 1960) because of the greater water solubility of the former. A small, but statistically significant increase in venous PCO<sub>2</sub> therefore reflects marked increases in tissue CO<sub>2</sub> reserve and metabolism (Dejours, 1963). Consequently, it cannot be excluded that donitriptan stimulates oxygen consumption and cerebral metabolism via 5-HT<sub>1B</sub> receptor activation, in addition to 5-HT<sub>1B</sub> receptor-mediated vasoconstriction of cranial AVAs. It was

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previously reported (Johnston & Saxena, 1978) that ergotamine evoked similar changes in blood gases to those presently described for donitriptan, in increasing jugular venous PO<sub>2</sub> and oxygen saturation, and with a notable propensity to increasing, albeit non-statistically significantly, PvCO<sub>2</sub> (Johnston & Saxena, 1978).

Such effects have not been previously described for triptans, but since most members of this drug class are also selective 5-HT<sub>1B/1D</sub> receptor agonists (De Vries *et al.*, 1999b; Goadsby, 1998), similar, but possibly less efficacious actions to those presently described for donitriptan can be expected (see below).

#### *Enhancement of tissue metabolism by donitriptan*

The mechanism by which donitriptan enhances cerebral metabolism clearly deserves further study. On the basis of the present results and current knowledge in the field, we tentatively propose the following scheme of events: 5-HT<sub>1B</sub> receptors have been demonstrated on endothelial cells of human brain arteries and microvessels (Nilsson *et al.*, 1999; Riad *et al.*, 1998; Van den Broek *et al.*, 2002b), but their precise function is unknown. Interestingly, endothelial cells are regulators of oxygen consumption via the cytochrome c oxidase oxygen sensor (Clementi *et al.*, 1999), and 5-HT<sub>1B</sub> receptor activation by sumatriptan and other triptans enhances cellular metabolic rate (Pauwels, 1998). Thus, by activating endothelial 5-HT<sub>1B</sub> receptors in the cerebral microcirculation, donitriptan could enhance oxygen consumption and tissue metabolism. This could provide an additional mechanism explaining, at least in part, the observed decreases in venous oxygen saturation and PO<sub>2</sub> and increases in PvCO<sub>2</sub>. Measurement of AVOSD in humans during migraine attacks before and after triptan administration could therefore test the clinical validity of the present data and provide further insight into the mechanism of acute antimigraine activity of triptans. The feasibility of such

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an approach has been demonstrated by Heyck (1969) in studies in which he measured AVOSD during attacks of migraine using the external jugular to obtain venous blood.

The current understanding of the chief mechanisms of triptan action in relieving migraine headache, based on 5-HT<sub>1B/1D</sub> receptor activation, comprises cranial vasoconstriction (De Vries *et al*, 1999a; Pauwels and John, 1999), peripheral (Moskowitz, 1992) and central (Hoskin *et al*, 1996) inhibition of trigeminal afferents thus preventing neurogenic plasma protein extravasation and sensory neuropeptide release (Goadsby and Knight, 1997; Limmroth *et al*, 2001). However, the present data suggest that donitriptan-evoked enhancement of cerebral oxygen utilisation and augmented tissue metabolism provides a further, important and complementary mechanism to cephalic AVA closure. It will be of particular interest to see whether the currently available triptans, which are considered to be partial agonists at 5-HT<sub>1B/1D</sub> receptors (John *et al.*, 1999; 2000), are as effective as donitriptan, a high efficacy 5-HT<sub>1B/1D</sub> receptor agonist, in respectively reducing and augmenting jugular venous oxygen saturation and PCO<sub>2</sub>, and enhancing cerebral metabolism.

In summary, donitriptan elicited selective carotid vasoconstriction and increases in AVOSD with identical potency in anesthetized pigs. Both actions were inhibited by GR 127935, indicating mediation by 5-HT<sub>1B</sub> receptors. Increases in AVOSD were mediated by decreases in jugular venous oxygen saturation and PO<sub>2</sub> and were unexpectedly accompanied by increases in PvCO<sub>2</sub>. The most plausible explanation for these effects with the notable exception of increased PvCO<sub>2</sub>, resides in closure of cephalic AVAs by donitriptan. However, a concomitant and complementary 5-HT<sub>1B</sub> receptor-mediated increase in cerebral oxygen extraction and enhancement of tissue metabolism is proposed as a further mechanism of action of donitriptan, which may be relevant to its migraine relieving efficacy.

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Figure 1:

Effects of donitriptan (filled symbols, n=7) on oxygen saturation in systemic arterial blood (AOS, circles) and oxygen saturation in jugular venous blood (VOS, squares) in the anesthetized pig. Data are means  $\pm$  SEM values of percent changes from baseline. \* P<0.05 *versus* vehicle-treated animals (open symbols, n=9) by one-way ANOVA followed by Dunnett's test.

Figure 2:

Maximal variations induced by donitriptan (solid bars, n=7 and 5 with GR 127935) or vehicle (open columns, n=9 and 6 with GR 127935) in arteriovenous difference in oxygen saturation (AVOSD, A panel) and in total carotid vascular resistance (TCVR, B panel) in the absence (left panel) and in presence of GR 127935 (0.63 mg/kg, right panel). Data are mean  $\pm$  SEM values of percent changes from baseline. \*: P<0.05 *versus* vehicle group by one-way ANOVA followed by Dunnett's test;

a: P<0.05 *versus* group without GR 127935 by one-way ANOVA followed by Dunnett's test.

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Table 1 :

Parameter	Treatment	Initial values	Absolute values						
			Dose ( $\mu\text{g/kg}$ ) of donitriptan						
			0.01	0.04	0.16	0.63	2.5	10	40
<b>PaO<sub>2</sub></b>	vehicle (n=9)	123 $\pm$ 6	121 $\pm$ 7	119 $\pm$ 7	119 $\pm$ 7	118 $\pm$ 7	116 $\pm$ 7	112 $\pm$ 6	114 $\pm$ 6
(mmHg)	donitriptan (n=7)	119 $\pm$ 7	119 $\pm$ 9	111 $\pm$ 10	116 $\pm$ 12	109 $\pm$ 13	100 $\pm$ 12	98 $\pm$ 12	98 $\pm$ 12
<b>PvO<sub>2</sub></b>	vehicle (n=9)	40.5 $\pm$ 2.2	41.3 $\pm$ 2.2	40.7 $\pm$ 2.3	39.6 $\pm$ 2.7	39.6 $\pm$ 2.0	39.0 $\pm$ 2.2	38.7 $\pm$ 2.6	39.4 $\pm$ 3.0
(mmHg)	donitriptan (n=7)	44.4 $\pm$ 10.5	45.5 $\pm$ 12.0	44.8 $\pm$ 11.4	43.7 $\pm$ 11.8	34.7 $\pm$ 5.0	31.1 $\pm$ 3.4	30.7 $\pm$ 3.0 *	30.7 $\pm$ 2.7 *
<b>Paco<sub>2</sub></b>	vehicle (n=9)	29.3 $\pm$ 3.5	30.4 $\pm$ 2.9	30.1 $\pm$ 3.0	30.5 $\pm$ 3.0	30.0 $\pm$ 3.2	29.5 $\pm$ 3.6	30.1 $\pm$ 3.6	29.5 $\pm$ 3.7
(mmHg)	donitriptan (n=7)	26.7 $\pm$ 1.2	26.3 $\pm$ 1.2	27.5 $\pm$ 1.3	27.7 $\pm$ 1.2	28.5 $\pm$ 1.0	28.8 $\pm$ 1.4	29.6 $\pm$ 1.4	29.7 $\pm$ 1.9
<b>Pvco<sub>2</sub></b>	vehicle (n=9)	37.9 $\pm$ 4.5	38.3 $\pm$ 4.0	37.0 $\pm$ 4.1	37.7 $\pm$ 4.0	38.4 $\pm$ 3.8	36.1 $\pm$ 3.8	37.4 $\pm$ 3.7	37.0 $\pm$ 3.8
(mmHg)	donitriptan (n=7)	35.4 $\pm$ 2.5	35.0 $\pm$ 2.6	35.9 $\pm$ 2.5	35.4 $\pm$ 2.4	37.8 $\pm$ 2.6	39.4 $\pm$ 2.6 *	41.6 $\pm$ 2.6 *	40.8 $\pm$ 2.3 *
<b>apH</b>	vehicle (n=9)	7.52 $\pm$ 0.03	7.52 $\pm$ 0.03	7.52 $\pm$ 0.03	7.53 $\pm$ 0.03	7.53 $\pm$ 0.03	7.54 $\pm$ 0.04	7.54 $\pm$ 0.03	7.54 $\pm$ 0.03
	donitriptan (n=7)	7.52 $\pm$ 0.02	7.52 $\pm$ 0.02	7.52 $\pm$ 0.02	7.52 $\pm$ 0.02	7.52 $\pm$ 0.02	7.52 $\pm$ 0.02	7.52 $\pm$ 0.02	7.53 $\pm$ 0.03
<b>vpH</b>	vehicle (n=9)	7.44 $\pm$ 0.03	7.45 $\pm$ 0.03	7.45 $\pm$ 0.03	7.46 $\pm$ 0.03	7.46 $\pm$ 0.03	7.46 $\pm$ 0.03	7.46 $\pm$ 0.03	7.46 $\pm$ 0.03
	donitriptan (n=7)	7.46 $\pm$ 0.02	7.46 $\pm$ 0.02	7.46 $\pm$ 0.02	7.46 $\pm$ 0.02	7.46 $\pm$ 0.02	7.46 $\pm$ 0.02	7.46 $\pm$ 0.03	7.46 $\pm$ 0.03
<b>AOS</b>	vehicle (n=9)	99.0 $\pm$ 0.4	99.0 $\pm$ 0.4	98.9 $\pm$ 0.4	98.8 $\pm$ 0.5	98.8 $\pm$ 0.5	98.7 $\pm$ 0.6	98.5 $\pm$ 0.6	98.6 $\pm$ 0.6
(%)	donitriptan (n=7)	100.1 $\pm$ 0.1	100.0 $\pm$ 0.3	99.5 $\pm$ 0.4	99.2 $\pm$ 0.8	98.6 $\pm$ 1.2	97.4 $\pm$ 2.0	96.8 $\pm$ 2.2	96.9 $\pm$ 2.1
<b>VOS</b>	vehicle (n=9)	68.8 $\pm$ 3.5	70.5 $\pm$ 3.7	70.9 $\pm$ 2.8	68.5 $\pm$ 4.2	69.9 $\pm$ 3.9	68.2 $\pm$ 3.8	67.7 $\pm$ 3.9	68.0 $\pm$ 3.7
(%)	donitriptan (n=7)	62.9 $\pm$ 9.1	62.1 $\pm$ 8.9	58.3 $\pm$ 9.1	55.4 $\pm$ 9.0	50.3 $\pm$ 8.5 *	44.2 $\pm$ 7.7 *	40.7 $\pm$ 7.3 *	41.2 $\pm$ 7.0 *
<b>AVOSD</b>	vehicle (n=9)	30.3 $\pm$ 3.2	28.4 $\pm$ 3.5	28.0 $\pm$ 2.7	30.2 $\pm$ 4.0	28.9 $\pm$ 3.6	30.5 $\pm$ 3.5	30.8 $\pm$ 3.7	30.6 $\pm$ 3.5
(%)	donitriptan (n=7)	37.3 $\pm$ 9.0	37.9 $\pm$ 8.8	41.2 $\pm$ 9.0	43.8 $\pm$ 8.8	48.3 $\pm$ 8.4 *	53.1 $\pm$ 7.3 *	56.1 $\pm$ 6.9 *	55.7 $\pm$ 6.8 *

Table 1:

Effects of donitriptan (n=7) and its vehicle (n=9) on blood gas parameters in the anesthetized pig. PaO<sub>2</sub>: partial pressure of oxygen in systemic arterial blood; PvO<sub>2</sub>: partial pressure of oxygen in jugular venous blood; Paco<sub>2</sub>: carbon dioxide partial pressure in systemic arterial

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blood; PvCO<sub>2</sub>: carbon dioxide partial pressure in jugular venous blood. apH: pH in systemic arterial blood. vpH: pH in jugular venous blood. AOS: oxygen saturation in systemic arterial blood; VOS: oxygen saturation in jugular venous blood and AVOSD: arteriovenous difference in oxygen saturation. Data are the means ± SEM in blood gas parameters following vehicle or donitriptan administration. \*: P<0.05 *versus* vehicle group assessed by one-way ANOVA with repeated measures followed by Student's t test.

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Table 2 :

Parameter (units)	Treatment	Initial values	Changes from initial values						
			Dose ( $\mu\text{g}/\text{kg}$ ) of donitriptan						
			0.01	0.04	0.16	0.63	2.5	10	40
<b>MAP</b> (mmHg)	vehicle (n=9)	87 $\pm$ 7	-2 $\pm$ 2	-1 $\pm$ 2	-3 $\pm$ 3	-2 $\pm$ 4	-3 $\pm$ 4	-2 $\pm$ 4	-2 $\pm$ 4
	donitriptan (n=7)	90 $\pm$ 3	1 $\pm$ 1	1 $\pm$ 1	5 $\pm$ 2	8 $\pm$ 3	12 $\pm$ 4 *	15 $\pm$ 4 *	13 $\pm$ 5 *
<b>HR</b> (beats/min)	vehicle (n=9)	126 $\pm$ 7	-7 $\pm$ 2	-12 $\pm$ 3	-14 $\pm$ 3	-17 $\pm$ 4	-19 $\pm$ 5	-20 $\pm$ 5	-19 $\pm$ 5
	donitriptan (n=7)	112 $\pm$ 5	-2 $\pm$ 3	-7 $\pm$ 3	-12 $\pm$ 4	-18 $\pm$ 5	-19 $\pm$ 5	-19 $\pm$ 6	-16 $\pm$ 6
<b>LVP</b> (mmHg)	vehicle (n=9)	100 $\pm$ 7	1 $\pm$ 5	0 $\pm$ 3	-1 $\pm$ 4	-2 $\pm$ 3	-4 $\pm$ 4	0 $\pm$ 4	-2 $\pm$ 4
	donitriptan (n=7)	105 $\pm$ 4	2 $\pm$ 2	-1 $\pm$ 2	3 $\pm$ 4	8 $\pm$ 4	12 $\pm$ 4 *	12 $\pm$ 5	12 $\pm$ 6
<b>Aortic BF</b> (ml/min)	vehicle (n=9)	1674 $\pm$	-67 $\pm$ 29	-99 $\pm$ 32	-142 $\pm$ 37	-163 $\pm$ 55	-169 $\pm$ 67	-196 $\pm$ 78	-197 $\pm$ 92
	donitriptan (n=7)	1875 $\pm$ 99	-106 $\pm$ 45	-348 $\pm$ 176	-310 $\pm$ 84	-404 $\pm$ 85 *	-429 $\pm$ 103 *	-458 $\pm$ 106	-426 $\pm$ 115
<b>TCBF</b> (ml/min)	vehicle (n=9)	148 $\pm$ 19	-1 $\pm$ 4	-1 $\pm$ 3	-2 $\pm$ 4	-2 $\pm$ 3	-1 $\pm$ 4	3 $\pm$ 5	3 $\pm$ 7
	donitriptan (n=7)	285 $\pm$ 33 *	0 $\pm$ 7	-10 $\pm$ 12	-27 $\pm$ 10 *	-80 $\pm$ 12 *	-120 $\pm$ 16 *	-139 $\pm$ 16 *	-143 $\pm$ 18 *
<b>LADBF</b> (ml/min)	vehicle (n=9)	24.1 $\pm$ 1.2	-0.8 $\pm$ 0.7	-2.3 $\pm$ 0.6	-3.4 $\pm$ 0.7	-3.9 $\pm$ 1.2	-4.1 $\pm$ 1.4	-4.4 $\pm$ 1.6	-4.8 $\pm$ 1.9
	donitriptan (n=7)	28.5 $\pm$ 3.2	0.1 $\pm$ 0.7	-0.8 $\pm$ 1.6	-2.9 $\pm$ 2.3	-3.4 $\pm$ 2.0	-4.3 $\pm$ 2.2	-4.2 $\pm$ 2.2	-3.7 $\pm$ 2.4

Table 2:

Effects of donitriptan (n=7) and its vehicle (n=9) on hemodynamic parameters in the anesthetized pig. Mean arterial pressure (MAP), heart rate (HR), left ventricular pressure (LVP), aortic blood flow (ABF), total carotid blood flow (TCBF) and left anterior descending coronary artery blood flow (LADBF) were measured. Data are the changes ( $\Delta$ )  $\pm$  SEM in hemodynamic parameters following vehicle or donitriptan administration. \*: P<0.05 *versus* vehicle group by one-way ANOVA followed by Student's t test.

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Figure 1:

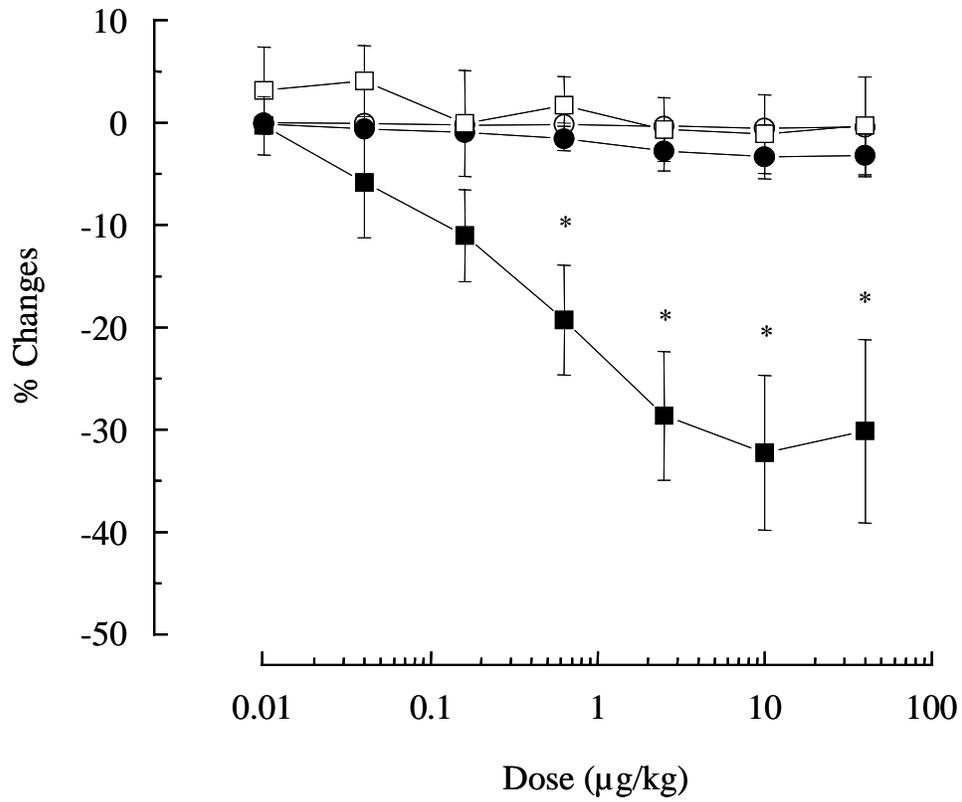


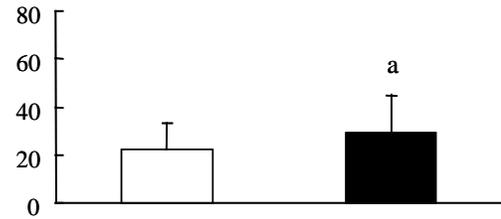
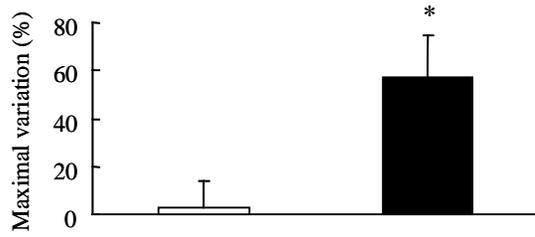
Figure 2:

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without GR 127935

with GR 127935

**A/ AVOSD**



**B/ TCVR**

