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The role of dopamine in a model of trigeminovascular nociception

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Running Title Page

Dopamine and the trigeminovascular system

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Section assignment: Neuropharmacology

Abbreviations

A68930 hydrochloride, cis-(±)-1-(Aminomethyl)-3,4-dihydro-3-phenyl-1H-2-benzopyran-5,6-diol hydrochloride

ANOVA, analysis of variance

CGRP, calcitonin gene-related peptide

S(-)-eticlopride hydrochloride, S(-)-3-Chloro-5-ethyl-N-([1-ethyl-2-pyrrolidinyl]methyl)-6-hydroxy-2-methoxybenzamide hydrochloride

L-745,870 hydrochloride, 3-([4-(4-chlorophenyl)piperazin-1-yl]methyl)-1H-pyrrolol(2,3-b)pyridine hydrochloride

(-)-quinpirole hydrochloride, (4aR*trans*)—4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1H-pyrazolol[3,4-g]quinoline hydrochloride

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R(+)-SCH-23390 hydrochloride, (*R*)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride

U99194A maleate, 5,6-Dimethyoxy-2-(di-n-propylamino)indan maleate

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Abstract

Migraine is a common disabling problem with three phases, premonitory, main headache attack and postdrome. The headache phase is believed to involve activation of trigeminal neurons, while the premonitory and postdrome phases may involve dopaminergic mechanisms. In animal studies, at very low doses dopamine has been found to cause vasodilation of cranial arteries. Using intravital microscopy we examined the effect of dopamine receptor agonists on dural blood vessel calibre, and the effect of dopamine and specific dopamine receptor antagonists on trigeminovascular neurogenic dural vasodilation. Dopamine hydrochloride caused a significant vasoconstriction ($P < 0.05$) and increase in arterial blood pressure ($P < 0.05$), that was reversed by a α_2-adrenoceptor antagonist, yohimbine, rather than specific dopamine receptor antagonists. The D_1 receptor agonist caused a vasoconstriction ($P < 0.05$) and a blood pressure increase ($P < 0.05$), which was reversed by yohimbine, and therefore α_2-adrenoceptor mediated. None of the specific dopamine receptor antagonists were able to attenuate neurogenic dural vasodilation. Dopamine hydrochloride infusion ($P < 0.05$) and a D_1 receptor agonist were able to attenuate the vasodilation ($P < 0.05$), maximal dilation returning after cessation of the dopamine agonist infusion. This response may be due to the vasoconstrictor effects of the α_2-adrenoceptor and an action at the D_1 receptor. In the intravital model of trigeminal activation it appears that dopamine receptors do not play a major role, and may not present an acute treatment option. Our data do not exclude a role for dopamine receptor modulators in short or long-term prevention.

Introduction

Migraine is a common, disabling neurological problem the precise pathogenesis of which remains to be delineated. It is likely that activation of trigeminal afferents innervating dural structures is involved in the headache phase of migraine (Goadsby et al., 2002). Stimulation of dural sites is painful in humans (Ray and Wolff, 1940) and activation of trigeminal neurons in animal studies causes calcitonin gene-related peptide (CGRP) release (Goadsby et al., 1988) and dural blood vessel dilation (Williamson et al., 1997b). Both the CGRP release (Goadsby and Edvinsson, 1993) and dural blood vessel dilation (Williamson et al., 1997b) are inhibited by the triptans, serotonin, 5-$HT_{1B/1D}$ receptor agonists, which are potent anti-migraine compounds (Ferrari et al., 2001). Recent clinical trial evidence suggests that blockade of CGRP receptors has a potent acute anti-migraine effect (Olesen et al., 2004).

As well as the headache phase in migraine there are also the premonitory and resolution phases, that are characterised by nausea, vomiting, hypotension and drowsiness, tiredness and mood changes, respectively (Headache Classification Committee of The International Headache Society, 2004). Given that these changes may be a result of monoamine, and specifically dopaminergic neurotransmission, dopamine has been implicated in migraine (Peroutka, 1997; Mascia et al., 1998; Fanciullacci et al., 2000). Additionally, migraine patients seem to show a hypersensitivity to dopamine agonists. Apomorphine, a dopamine receptor agonist, produces more yawning in migraineurs than in age-matched controls (Blin et al., 1991) and piribedil caused increase in cerebral blood flow, as well as inducing nausea, vomiting and hypotension that was blocked by the peripheral D_2-receptor antagonist domperidone (Bes et al., 1986). Two small studies have shown

domperidone can prevent the occurrence of migraine if it is taken during the premonitory phase (Amery and Waelkens, 1983; Waelkens, 1984). This has led to a dopamine theory of migraine.

Studies performed on cat pial arteries *in vivo* and middle cerebral arteries *in vitro* showed that at very low doses dopamine agonists caused slight vasodilation, while at higher doses dopamine caused vasoconstriction (Edvinsson et al., 1978a; Edvinsson et al., 1978b). Similarly, dopamine and apomorphine intra-carotid infusions caused a dose-dependent vasoconstriction in dog (Villalon et al., 2003), although they found that a selective D_1-receptor agonist caused slight vasodilation, as did the effects of dopamine after it was antagonised by a α_2-adrenergic receptor antagonist.

Given the effects of dopamine on the cerebral, pial and carotid arteries, as well as the renal and mesenteric vasculature we wanted to examine what effects dopamine might have on the dural vasculature, and therefore, whether it may be involved in any direct way, in the headache phase of migraine. The intravital microscopy model of trigeminovascular activation utilises the reaction of meningeal blood vessel calibre after electrical stimulation of a cranial window, as a model of trigeminal nerve fibre activation (Williamson et al., 1997b), and it has proved to be an excellent model in predicting anti-migraine efficacy (Williamson et al., 1997b). Previously the triptans have been shown to attenuate neurogenic dural vasodilation (Williamson et al., 1997b). We looked at the effects of dopamine agonists and antagonists on neurogenic dural vasodilation. We also looked at the direct effects of dopamine and specific D_1 and D_2 receptor agonists on dural blood vessel calibre. The response of dopamine was also challenged with specific dopamine receptor antagonists, as well as α_2-

adrenoceptor antagonists. We monitored carefully any changes in arterial blood pressure related to dopamine and the various antagonists.

Materials and Methods

Surgical Preparation

All experiments were conducted under the UK Home Office (Scientific Procedures) Act (1986). Male Sprague-Dawley rats (280-385 g) were anaesthetised throughout the experiments with sodium pentobarbitone (60mgkg^{-1} i.p. initially and then 18mgkg^{-1}hr- i.v. infusion). The left femoral artery and vein were cannulated for blood pressure recording and intravenous infusion of anaesthetic, respectively. Temperature was maintained throughout using a homeothermic blanket system. The rats were placed in a stereotaxic frame, the skull exposed and the right or left parietal bone thinned by drilling with a saline-cooled drill until the blood vessels of the dura mater were clearly visible through the intact skull.

Intravital Microscopy

The cranial window was covered with mineral oil (37^{o}C) and a branch of the middle meningeal artery viewed using an intravital microscope (Microvision MV2100, UK) and the image displayed on a television monitor. Dural blood vessel diameter was continuously measure using a video dimension analyser (Living Systems Instrumentation, USA) and displayed with blood pressure on a data analysis system (Spike2 v4, Cambridge Electronic Design, Cambridge UK).

Experimental Protocols

Defining electrical stimulation parameters. Electrical stimulation was used to evoke neurogenic dural vasodilation with a bipolar stimulating electrode (NE 200X, Clark Electromedical) that was placed on the surface of the cranial window approximately 200μm from the vessel of interest. The surface of the cranial window was stimulated

at 5 Hz, 1ms for 10 seconds (Grass Stimulator S88, Grass Instrumentation, MA) with increasing voltage until maximal dilation was observed. Subsequent electrically induced responses in the same animal were then evoked using the same voltage.

Effect of dopamine receptor agonists and dopamine antagonists on neurogenic dural vasodilation

The effect of dopamine hydrochloride and the specific dopamine receptor agonists, A68930 hydrochloride and (-)-quinpirole hydrochloride on neurogenic dural vasodilation were studied. Dopamine (20 or 40 $\mu gkg^{-1}min^{-1}$), A68930 hydrochloride (50 $\mu gkg^{-1}min^{-1}$) and (-)-quinpirole hydrochloride (50 $\mu gkg^{-1}min^{-1}$) were intravenously infused at least ten minutes after a control response to electrical stimulation. The electrical stimulation was then repeated five minutes into the infusion and the infusion continued for a further five minutes. At least ten minutes after the completion of the dopamine infusion, the electrical stimulation was again repeated. The response of dopamine hydrochloride on neurogenic dural vasodilation was also challenged with the α_2-adrenoceptor antagonist, yohimbine (3 $mgkg^{-1}$), the D_1 receptor antagonist, R(+)-SCH-23390 (1.0 $mgkg^{-1}$) and the D_2 receptor antagonist, S(-)-eticlopride hydrochloride (3.0 $mgkg^{-1}$). Similarly the response of A68930 hydrochloride on neurogenic dural vasodilation was also challenged with yohimbine (3 $mgkg^{-1}$).

In a separate series of experiments the neurogenic dural vasodilator response was challenged with a D_1 dopamine receptor antagonist, R(+)-SCH-23390. A control response to electrical stimulation was followed at least ten minutes later by an intravenous bolus of R(+)-SCH-23390 (0.3 $mgkg^{-1}$) and followed five minutes later

by a repeat electrical stimulation. This protocol was repeated for an increased dose of R(+)-SCH-23390 (1.0 $mgkg^{-1}$) in the same animal. A similar series of experiments were also completed with a D_2 dopamine receptor antagonist, S(-)-eticlopride hydrochloride (0.3, 1.0 and 3.0 $mgkg^{-1}$), a D_3 dopamine receptor antagonist, U99194A maleate (0.3, 1.0 and 3.0 $mgkg^{-1}$) and a D_4 dopamine receptor antagonist, L-745,870 hydrochloride (0.3, 1.0 and 3 $mgkg^{-1}$).

Effects of dopamine receptor agonists and antagonists on dural blood vessel calibre

The effect of dopamine hydrochloride, the D_1 receptor agonist, A68930 hydrochloride and the D_2 receptor agonist, (-)-quinpirole, on dural blood vessel diameter was studied. Increasing doses of dopamine hydrochloride (0.5, 1, 2, 5, 10, 15, 20 and 40 $\mu gkg^{-1}min^{-1}$), A68930 hydrochloride (1, 10 and 50 $\mu gkg^{-1}min^{-1}$) and (-)-quinpirole (1, 10 and 50 $\mu gkg^{-1}min^{-1}$) were administered as an infusion for ten minutes each, with a gap of five minutes between each increase of dose. In a separate series of experiments the effects of dopamine hydrochloride were challenged with a D_1 dopamine receptor antagonist, R(+)-SCH-23390. Dopamine was infused at 40 $\mu gkg^{-1}min^{-1}$ for ten minutes, and then after a further ten minutes R(+)-SCH-23390 (0.3 $mgkg^{-1}$) was administered, five minutes later a ten minute dopamine infusion was repeated. A further ten minutes after the dopamine infusion was completed an increased dosage of R(+)-SCH-23390 (1.0 $mgkg^{-1}$) was administered and the dopamine infusion repeated. A similar series of experiments were also completed with a D_2 dopamine receptor antagonist, S(-)-eticlopride hydrochloride (0.3, 1.0 and 3.0 $mgkg^{-1}$), a D_3 dopamine receptor antagonist, U99194A maleate (0.3, 1.0 and 3.0 $mgkg^{-1}$) and a D_4 dopamine receptor antagonist, L-745,870 hydrochloride (0.3, 1.0 and 3 $mgkg^{-1}$).

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In a separate series of experiments we examined the response of 40 $\mu gkg^{-1}min^{-1}$ dopamine hydrochloride infusion with yohimbine, the α_2-adrenergic receptor antagonist. Using the protocol above mentioned, 40 $\mu gkg^{-1}min^{-1}$ dopamine was infused for ten minutes, and was followed by yohimbine (3 $mgkg^{-1}$) which was followed five minutes later by a repeat of the dopamine infusion. A68930 hydrochloride (50 $\mu gkg^{-1}min^{-1}$) and (-)-quinpirole (50 $\mu gkg^{-1}min^{-1}$) were also both challenged with yohimbine (3 $mgkg^{-1}$).

Data Analysis

The peak effects of electrical stimulation and dopamine infusion on dural vessel diameter was calculated as a percentage change from the pre-stimulation baseline diameter. The nature of the experimental set-up, where the magnification of the dural vessel selected for study was different in each set-up made it impractical to standardise the dural vessel measurement, therefore, the dural vessel diameter was measured in arbitrary units, and all calculations related to the pre-manipulation baseline. The vessel size was approximately 150-200 μm. All data are expressed as mean ± SEM. Statistical analysis was performed using an ANOVA for repeated measures with Bonferroni *post-hoc* correction for multiple comparisons followed by Student's paired *t*-test where appropriate (SPSS v10.0). Significance was assessed at the $P < 0.05$ level or below.

The reproducibility of the neurogenic vasodilator response has been tested previously using four consecutive saline-controlled stimuli (Akerman et al., 2002) in the same experimental set-up.

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Drugs

Dopamine was purchased as dopamine hydrochloride pre-dissolved in water for injection (Faulding Pharmaceuticals Plc, UK). (*R*)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (R(+)-SCH-23390 hydrochloride, in a 10 $mgml^{-1}$ solution), S(-)-3-Chloro-5-ethyl-N-([1-ethyl-2-pyrrolidinyl]methyl)-6-hydroxy-2-methoxybenzamide hydrochloride (S(-)-eticlopride hydrochloride, in a 10 $mgml^{-1}$ solution), 5,6-Dimethyoxy-2-(di-n-propylamino)indan maleate (U99194A maleate, in a 10 $mgml^{-1}$ solution) and 3-([4-(4-chlorophenyl)piperazin-1-yl]methyl)-1H-pyrrolol(2,3-b)pyridine hydrochloride (L-745,870 hydrochloride, in a 10 $mgml^{-1}$ solution) (all Sigma-Aldrich, UK) were dissolved in water for injection as salts and administered in an approximate volume of 0.3 ml. cis-(±)-1-(Aminomethyl)-3,4-dihydro-3-phenyl-1H-2-benzopyran-5,6-diol hydrochloride (A68930 hydrochloride) and (4aR*trans*)—4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1H-pyrazolol[3,4-g]quinoline hydrochloride ((-)-quinpirole hydrochloride) (Tocris Cookson Ltd, UK) were dissolved in water for injection. There is a summary of drugs used in table 1.

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Results

Effects of dopamine and dopamine receptor antagonist of neurogenic dural vasodilation

Neurogenic dural vasodilation with electrical stimulation was significantly inhibited when compared to control during infusion of 20 $\mu gkg^{-1}min^{-1}$ dopamine, 116.7 ± 14 % to 46 ± 9 % (n = 11, t_{10} = 6.78, $P < 0.05$), an uninhibited vasodilation was restored post dopamine infusion, 46 ± 9 % to 111.2 ± 8 % (n = 11, t_{10} = -7.98, $P < 0.05$). This is similarly the case for the 40 $\mu gkg^{-1}min^{-1}$ dopamine infusion, vasodilation was inhibited from 145.1 ± 15 % to 76.3 ± 12 % (n = 7, t_6 = 3.92, $P < 0.05$), and the full vasodilation was restored post dopamine infusion, 76.3 ± 12 % to 137.5 ± 20 % (n = 7, t_6 = 2.93, $P < 0.05$; Figure 1A). Specific dopamine receptor agonists were also infused, A68930 hydrochloride (50 $\mu gkg^{-1}min^{-1}$) was able to significantly inhibit neurogenic dural vasodilation from 102.0 ± 8 % to 42.3 ± 14 % (n = 6, t_5 = 3.46, $P < 0.05$) and the neurogenic dural vasodilation response returned post A68930 hydrochloride infusion, 42.3 ± 14 % compared to 93.0 ± 14 % (n = 6, t_5 = -2.82, $P < 0.05$). The neurogenic dural vasodilator response after (-)-quinpirole hydrochloride (50 $\mu gkg^{-1}min^{-1}$) was not significant, 97.3 ± 9 % compared to 94.5 ± 13 % (n = 6, t_5 = 0.43, P = 0.685).

The dopamine hydrochloride induced inhibition of neurogenic dural vasodilation was blocked by pre-treatment with the α_2-adrenergic receptor antagonist yohimbine (3 $mgkg^{-1}$), 31.4 ± 8 % compared to 72.5 ± 8 % (n = 6, t_5 = 2.26, P = 0.073), and by the D_1 dopamine receptor antagonist R(+)-SCH-23390 (1 $mgkg^{-1}$), 31.4 ± 8 % compared to 54.8 ± 10 % (n = 6, t_5 = 4.4, $P < 0.05$), although in each case the response was still

significantly different. The dopamine hydrochloride response was not inhibited by the specific D_2 dopamine receptor antagonist S(-)-eticlopride hydrochloride (3 $mgkg^{-1}$), 31.4 ± 8 % compared to 35.0 ± 6 % (n = 6, t_5 = 9.99, P < 0.05), see figure 1B. The A68930 hydrochloride induced inhibition of neurogenic dural vasodilatation was also reversed by yohimbine (3 $mgkg^{-1}$), 24.49 ± 9 % compared to 102.6 ± 4 % (n = 5, t_4 = -6.15, P < 0.05), see figure 2.

None of the dopamine receptor antagonists were able to inhibit neurogenic dural vasodilation at the doses applied, data are summarised in table 2 and figure 3. The effects of the dopamine antagonists themselves on dural blood vessel diameter and mean arterial blood pressure are summarised in table 4. In each case where significant changes in dural blood vessel diameter occurred, the baseline vessel diameter was restored naturally before a repeat electrical stimulation.

Effect of dopamine hydrochloride and D_1 and D_2 receptor agonists on dural blood vessel diameter and mean arterial blood pressure

In rats treated with dopamine hydrochloride (0.5, 1, 2, 5, 10, 15, 20 and 40 $\mu gkg^{-1}min^{-1}$), there was no significant effect on dural vessel diameter across all doses using an ANOVA for repeated measures (n = 6, $F_{7,35}$ = 1.423, P = 0.288), although using a Student's paired t-test both the 20 (t_{16} = 3.8, P < 0.05) and 40 $\mu gkg^{-1}min^{-1}$ (t_{39} = 7.9, P < 0.05) doses were significant, in all animals tested (Table 3). The slight change in dural blood vessel diameter was accompanied by a significant increase in blood pressure across all doses (n = 6, $F_{7,35}$ = 16.667, P < 0.0001). The blood pressure change at the 15 $\mu gkg^{-1}min^{-1}$ (t_5 = 3.427, P < 0.05), 20 $\mu gkg^{-1}min^{-1}$ (t_{16} = -6.15, P <

0.05) and 40 μgkg^{-1}min^{-1} (t_{39} = -11.62, P < 0.05) doses were significant when compared to the pre-injection blood pressure (Table 3).

A68930 hydrocloride (1, 10 and 50 μgkg^{-1}) did not cause any significant change to the dural blood vessel diameter across the cohort (n = 6, $F_{2,10}$ = 2.3, P = 0.175), however using Student's paired t-test both 10 (t_5 = 4.5, P < 0.05) and 50 μgkg^{-1} (t_{13} = 3.85, P < 0.05) proved significant in all animals tested. There was a significant change in arterial blood pressure overall (n = 6, $F_{2,10}$ = 27.6, P < 0.05). The blood pressure change with the 50 μgkg^{-1} dose of A68930 hydrochloride proved to be significant (t_{13} = -7.88, P < 0.05). (-)-Quinpirole hydrochloride (1, 10 and 50 μgkg^{-1}) did not cause any significant change in both dural blood vessel diameter (n = 6, $F_{2,10}$ = 0.381, P = 0.63) or arterial blood pressure (n = 6, $F_{2,10}$ = 2.78, P = 0.137), these data are summarised in table 4.

Effects of dopamine receptor antagonists and a α_2-adrenergic receptor antagonist on blood vessel diameter and mean arterial blood pressure changes caused by dopamine

The effects of 40 μgkg^{-1}min^{-1} dopamine were challenged with various dopamine receptor antagonists. When the effects of 40 μgkg^{-1}min^{-1} dopamine on vessel diameter were compared to the pre-injection diameter, there was a significant drop in diameter (t_{24} = 5.619, P < 0.001, n = 25). R(+)-SCH-23390, the D_1 receptor antagonist had no significant effect on the dopamine-induced changes to dural blood vessel diameter ($F_{2,10}$ = 1.0, P = 0.37, n = 6). The dopamine-induced blood pressure changes were significantly reduced with R(+)-SCH-23390 when compared to the control response ($F_{2,10}$ = 6.451, P = 0.029, n = 6). With the 1.0 mgkg^{-1} dose of R(+)-SCH-23390, there

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was a 46.2 ± 6 mmHg compared to 34.1 ± 6 mmHg decrease in mean arterial blood pressure (t_5 = 3.05, P = 0.028, n = 6).

S(-)-eticlopride hydrochloride, the D_2 receptor antagonist had no significant effect on the dopamine-induced changes to dural blood vessel diameter ($F_{3,18}$ = 0.282, P = 0.682, n = 7) or mean arterial blood pressure ($F_{3,18}$ = 3.857, P = 0.06, n = 7).

U99194A maleate, the D_3 receptor antagonist had no significant effect on the dopamine-induced changes to dural blood vessel diameter ($F_{2,10}$ = 1.404, P = 0.292, n = 6) or mean arterial blood pressure ($F_{2,10}$ = 1.401, P = 0.292, n = 6). Finally, in rats treated with L-745,870 hydrochloride, the D_4 receptor antagonist, there was no significant effect on the dopamine-induced changes to dural blood vessel diameter ($F_{2,12}$ = 0.279, P = 0.719, n = 7) or mean arterial blood pressure ($F_{2,12}$ = 0.714, P = 0.448, n = 7).

Yohimbine (3 $mgkg^{-1}$), the α_2-adrenergic receptor antagonist, was able to significantly attenuate the dural blood vessel changes caused by 40 $\mu gkg^{-1}min^{-1}$ dopamine infusion, from a 22.2 ± 4% to a 3.3 ± 2% reduction in vessel diameter (n = 7, t_6 = 4.73, P < 0.05). The mean arterial blood pressure changes caused by dopamine infusion were also significant reduced from 29.3 ± 7 mmHg to 2.7 ± 2 mmHg (n = 7, t_6 = 3.54, P < 0.05).

The effects of the D_1 and D_2 dopamine receptor agonists were also challenged with yohimbine (3 $mgkg^{-1}$). Yohimbine did not alter the response of A68930 hydrochloride (50 $\mu gkg^{-1}min^{-1}$) on dural blood vessel diameter (t_5 = 1.42, P = 0.214, n = 6), but was able to reverse the arterial blood pressure effects from a 40.2 ± 4 mmHg increase to a 10.7 ± 3 mmHg increase (t_5 = 5, P < 0.05, n = 6). Yohimbine did not alter the response of (-)-quinpirole hydrochloride (50 $\mu gkg^{-1}min^{-1}$) on dural blood vessel

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diameter (t_4 = 0.23, P = 0.828, n = 5) and arterial blood pressure (t_4 = 0.19, P = 0.86, n = 5; Table 4).

Effects of dopamine antagonists and a α_2-adrenerngic receptor antagonist on dural blood vessel diameter and mean arterial blood pressure

The data for the changes caused by the dopamine receptor antagonists on dural blood vessel diameter and mean arterial blood pressure are summarised in table 5. Briefly, the D_1 receptor antagonist, R(+)-SCH-23390, significantly increased blood pressure and this was accompanied by a significant decrease in dural vessel diameter. S(-)-eticlopride hydrochloride, the D_2 receptor antagonist only caused a significant change in blood pressure at the 3 $mgkg^{-1}$ dose, and there was no significant change to dural vessel diameter. The D_3 receptor antagonist, U99194A maleate significantly increased blood pressure at all doses, but only altered dural vessel diameter at the 1.0 $mgkg^{-1}$ dose. Finally L-745,870 hydrochloride, the D_4 receptor antagonist significantly decreased blood pressure at the 1 and 3 $mgkg^{-1}$ dose, but only effected the dural blood vessel diameter at the 1 $mgkg^{-1}$ dose.

Yohimbine, the α_2-adrenergic receptor antagonist, caused a significant reduction in mean arterial blood pressure of 34.4 ± 5 mmHg (n = 13, t_{12} = 7.51, $P < 0.05$), this was accompanied by a significant 52.4 ± 18% increase (n = 13, t_{12} = 3.41, $P < 0.05$) in dural blood vessel diameter. Both were naturally restored to their pre-injection levels within the time constraints of the experimental protocol.

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Discussion

There are five different dopamine receptor subtypes thus far identified (D_1, D_2, D_3, D_4 and D_5), classified as D_1-like (D_1 and D_5), which are positively coupled to adenylyl cyclase and D_2-like (D_2, D_3 and D_4) which are negatively coupled to adenylyl cyclase (Missale et al., 1998). Given that apomorphine, the dopamine receptor agonist, exacerbates yawning during the migraine attack (Blin et al., 1991), and domperidone, the D_2 dopamine receptor antagonist, blocked nausea and vomiting caused by piribedil (Bes et al., 1986), we chose to antagonise dopamine receptors during neurogenic dural vasodilation. The D_1, D_2, D_3 and D_4 dopamine receptors were all unable to inhibit or attenuate neurogenic dural vasodilation.

Administration of dopamine hydrochloride and A68930 hydrochloride, the D_1 receptor agonist, we were able to attenuate neurogenic dural vasodilation. Upon cessation of dopamine agonist infusion, repeat electrical stimulation was able to produce a maximum neurogenic vasodilation. In each case this effect was partly antagonised by the α_2-adrenoceptor antagonist, yohimbine. The dopamine hydrochloride response was also partially attenuated by the D_1 receptor antagonist, SCH23390, although the antagonised response was still significantly less than the control neurogenic dural vasodilation. The D_2 receptor agonist had no effect on neurogenic dural vasodilation. The ability of dopamine hydrochloride to attenuate neurogenic dural vasodilation may in part be explained by the vasoconstrictive effect of dopamine, which we believe to be caused as a response to the profound blood pressure changes. We have established already, and it has been shown previously, the vasoconstriction and blood pressure changes appear to be mediated by a

noradrenergic response at the α_2-adrenoceptor (Edvinsson et al., 1978a; Edvinsson et al., 1978b; Willems et al., 1999; Villalon et al., 2003). It therefore appears that some of the inhibition is mediated by the α_2-adrenoceptor. It is possible that the D_1 dopamine receptor may contribute to this effect given the response of the agonist and antagonist in this model. The D_1 dopamine receptor antagonist was unable to fully inhibit the effects of the dopamine induced inhibition, therefore, we describe a clear but partial response. The D_1 receptor agonist's attenuation of neurogenic dural vasodilation was almost fully inhibited by α_2-adrenoceptor modulation, therefore any action of dopamine agonists will be compromised by α_2-adrenoceptor activation and blood pressure effects. The partial D_1 dopamine receptor component in this model of trigeminovascular nociception may explain the actions of some migraine preventives that are known to act on dopamine receptors (Peroutka, 1997; Mascia et al., 1998; Fanciullacci et al., 2000).

Neurogenic dural vasodilation is believe to result from the pre-synaptic release of CGRP from trigeminal nerve terminals acting on CGRP receptors on the dural blood vessels causing vasodilation (Williamson et al., 1997b). The data presented suggest that D_1 dopamine receptors may be involved in the control of the dural vasculature on trigeminal nerve endings. It has been shown previously that there is a lack of response on neurogenic dural vasodilation when α_2-adrenoceptors are manipulated (Akerman et al., 2001), therefore it seems unlikely that dopamine is activating through pre-junctional α_2-adrenoceptors to exert its inhibitory action in the trigeminovascular system. There is evidence that D_2 dopamine receptors are present in the trigeminal ganglion, using a cDNA probe and hybridisation techniques (Peterfreund et al., 1995), although from the data they may not be transported to peripheral trigeminal nerve

endings, so that antagonising these receptors did not affect Aδ-trigeminal nerve activation and neurogenic dural vasodilation. It seems that the majority of the inhibitory response is mediated by the α_2-adrenoceptor effect on mean arterial blood pressure, but there is a minor, significant response mediated by D_1 dopamine receptors.

The use of yohimbine as the α_2-adrenoceptor antagonist may seem odd given its action at other receptors relevant in this system, 5-$HT_{1A/1B/1D}$ agonist and D_2 and D_3 antagonist (Millan et al., 2000). However, we have shown previously that the α_2-adrenoceptor is not involved in neurogenic dural vasodilation (Akerman et al., 2001). 5-$HT_{1B/1D}$ receptors have been shown previously to inhibit the neurogenic response (Williamson et al., 1997b), the dose of yohimbine is not sufficient to inhibit these neurons. Yohimbine is able to actively inhibit α_2-adrenoceptor agonist effects at this dose (Hsu and Kakuk, 1984; Liu and Coupar, 1997). There is little evidence of a 5-HT_{1A} effect in the trigeminovascular system (Cumberbatch et al., 1998). Finally from evidence taken from the data presented here, neither D_2 nor D_3 receptors have any effect on either dopamine induced vasoconstriction changes or on neurogenic dural vasodilation. It was also found that D_2 receptor antagonists were unable to reverse the dopamine inhibition of neurogenic dural vasodilation, and given that D_3 receptors are considered D_2-like we feel we can tentatively conclude that yohimbine effects are α_2-adrenoceptor specific.

As reported in pial, cerebral and carotid arteries (Edvinsson et al., 1978a; Edvinsson et al., 1978b; Villalon et al., 2003), increasing doses of dopamine caused a vasoconstriction in the dural meningeal arteries. This was significant at the highest

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doses given in this study, but other studies have used higher dosing regimens. We were primarily interested in a vasodilatory response, and only found a significant vasodilation at the 1 $\mu gkg^{-1}min^{-1}$ dose regimen. We observe that at a lower dose there was no significant dilation and at higher doses we found increasing vasoconstriction, similar to other studies.

It was unexpected that the vasodilatory effect was not clearly dose-dependent, and the vasodilation was not as extensive as that found in other *in vivo* studies. Indeed vasodilation has been found at much higher doses, also using an intravenous method of entry (Villalon et al., 2003; Polakowski et al., 2004). Villalon *et al* (2003) only found a vasodilator effect with dopamine hydrochloride in the presence of a α_2-adrenoceptor antagonist, we saw no vasodilator effect in the presence of yohimbine in the dural circulation. Previous studies have also shown that a specific D_1 receptor agonist, fenoldopam, was able to cause vasodilation on its own (Villalon et al., 2003; Polakowski et al., 2004). In our study the specific D_1 receptor agonist caused a significant vasoconstriction in dural blood vessel calibre and a significant increase in arterial blood pressure; these changes were reversed by a α_2-adrenoceptor antagonist. The D_2 receptor agonist was unable to significantly alter either blood vessel diameter or mean arterial blood pressure. We conclude that the changes observed in the dural blood vessels and arterial blood pressure are mediated by vascular α_2-adrenoceptors rather than dopamine receptors.

Dopamine acts as a precursor to the catecholamines noradenaline and adrenaline, which mediate vasoconstriction and blood pressure increase through the α_1 and α_2–adrenoceptors (Willems et al., 1999). In the present study dopamine is likely to be

acting as a precursor to noradrenaline in this biological system and thus activating the noradrenergic system to cause vasoconstriction and blood pressure increase. Only the 1 $mgkg^{-1}$ dose of the D_1 dopamine receptor antagonist was able to attenuate the blood pressure increase, but there was still a significant blood pressure increase, the α_2-adrenoceptor antagonist was able to reverse both the vasoconstriction and the blood pressure changes caused by the highest dose of dopamine hydrochloride.

The action of the dopamine agonist fenoldopam caused mean arterial blood pressure decrease accompanied by a vasodilation (Polakowski et al., 2004), which conflicts with the findings of A68930 hydrochloride used in the present study, which showed only vasoconstriction and was inhibited by the α_2-adrenoceptor antagonist. This is similar to the effects of dopamine hydrochloride. The differences in the response of the specific dopamine agonists may represent a difference in their abilities to activate noradrenergic production, it would certainly be interesting to observe the affects of fenoldopam in the system used in this study.

Significant blood pressure changes were caused by all dopamine receptor antagonists at varying doses, and these were variously accompanied by a change in dural blood vessel diameter. Given the role of dopamine as a precursor of both noradrenaline and adrenaline, and given that amines are released to maintain vascular tone, it is possible that the effect of the dopamine antagonists on blood pressure are a response to inhibition of the precursor to adrenergic synthesis, namely dopamine, and the dural blood vessel diameter changes are a response to the change in blood pressure. This seems to be indicated by the lack of inhibitory effect of the dopamine receptor

antagonists on dopamine hydrochloride induced changes, while the α_2-adrenoceptor antagonist had profound inhibitory effects.

Despite the evidence that dopamine receptors may be present in the trigeminovascular system (Peterfreund et al., 1995), and that dopamine agonists have been found to exacerbate certain types of headache (Levy et al., 2003), only the D_1 receptor is able to attenuate or inhibit the activation of dural blood vessels or trigeminal neurons, and this response was only a partial effect, suggesting perhaps a small role in the acute phase of migraine. There were other effects, perhaps due to vasoconstriction caused by activation of the α_2-adrenoceptor. The involvement of dopamine and its receptors in migraine may dominate in other aspects of the attack, such as the initiation, providing a role for dopamine modulators in short or long-term prevention.

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References

Akerman S, Williamson DJ, Hill RG and Goadsby PJ (2001) The effect of adrenergic compounds on neurogenic dural vasodilatation. *European Journal of Pharmacology* **424**:53-58.

Akerman S, Williamson DJ, Kaube H and Goadsby PJ (2002) Nitric oxide synthase inhibitors can antagonize neurogenic and calcitonin gene-related peptide induced dilation of dural meningeal vessels. *Br J Pharmacol* **137**:62-68.

Amery WK and Waelkens J (1983) Prevention of the last chance: an alternative pharmacologic treatment of migraine. *Headache* **23**:37-38.

Bardo MT, Valone JM and Bevins RA (1999) Locomotion and conditioned place preference produced by acute intravenous amphetamine: role of dopamine receptors and individual differences in amphetamine self-administration. *Psychopharmacology (Berl)* **143**:39-46.

Bes A, Dupui P, Guell A, Bessoles G and Geraud G (1986) Pharmacological exploration of dopamine hypersensitivity in migraine patients. *Int J Clin Pharmacol Res* **6**:189-192.

Blin O, Azulay JP, Masson G, Aubrespy G and Serratrice G (1991) Apomorphine-induced yawning in migraine patients: enhanced responsiveness. *Clin Neuropharmacol* **14**:91-95.

Clifford JJ and Waddington JL (1998) Heterogeneity of behavioural profile between three new putative selective D3 dopamine receptor antagonists using an ethologically based approach. *Psychopharmacology (Berl)* **136**:284-290.

Cumberbatch MJ, Hill RG and Hargreaves RJ (1998) The effects of 5-HT1A, 5-HT1B and 5-HT1D receptor agonists on trigeminal nociceptive neurotransmission in anaesthetized rats. *Eur J Pharmacol* **362**:43-46.

DeNinno MP, Schoenleber R, MacKenzie R, Britton DR, Asin KE, Briggs C, Trugman JM, Ackerman M, Artman L and Bednarz L (1991) A68930: a potent agonist selective for the dopamine D1 receptor. *Eur J Pharmacol* **199**:209-219.

Edvinsson L, Hardebo JE, McCulloch J and Owman C (1978a) Effects of dopaminergic agonists and antagonists on isolated cerebral blood vessels. *Acta Physiol Scand* **104**:349-359.

Edvinsson L, Hardebo JE, McCulloch J and Owman C (1978b) Vasomotor response of cerebral blood vessels to dopamine and dopaminergic agonists. *Adv Neurol* **20**:85-96.

Fanciullacci M, Alessandri M and Del Rosso A (2000) Dopamine involvement in the migraine attack. *Funct Neurol* **15 Suppl 3**:171-181.

Ferrari MD, Roon KI, Lipton RB and Goadsby PJ (2001) Oral triptans (serotonin 5-HT(1B/1D) agonists) in acute migraine treatment: a meta-analysis of 53 trials. *Lancet* **358**:1668-1675.

Goadsby PJ and Edvinsson L (1993) The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. *Ann Neurol* **33**:48-56.

Goadsby PJ, Edvinsson L and Ekman R (1988) Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system. *Ann Neurol* **23**:193-196.

Goadsby PJ, Lipton RB and Ferrari MD (2002) Migraine--current understanding and treatment. *N Engl J Med* **346**:257-270.

Headache Classification Committee of The International Headache Society (2004) The International Classification of Headache Disorders (second edition). *Cephalalgia* **24**:1-160.

Hsu WH and Kakuk TJ (1984) Effect of amitraz and chlordimeform on heart rate and pupil diameter in rats: mediated by alpha 2-adrenoreceptors. *Toxicol Appl Pharmacol* **73**:411-415.

Kawashima N, Okuyama S, Omura T, Chaki S and Tomisawa K (1999) Effects of selective dopamine D4 receptor blockers, NRA0160 and L-745,870, on A9 and A10 dopamine neurons in rats. *Life Sci* **65**:2561-2571.

Kebabian JW, Briggs C, Britton DR, Asin K, DeNinno M, MacKenzie RG, McKelvy JF and Schoenleber R (1990) A68930: a potent and specific agonist for the D-1 dopamine receptor. *Am J Hypertens* **3**:40S-42S.

Levy MJ, Matharu MS and Goadsby PJ (2003) Prolactinomas, dopamine agonists and headache: two case reports. *Eur J Neurol* **10**:169-173.

Liebman JM, Gerber R, Hall NR and Altar CA (1988) Heterogeneous rotational responsiveness in 6-hydroxydopamine-denervated rats: pharmacological and neurochemical characterization. *Psychopharmacology (Berl)* **96**:477-483.

Liu L and Coupar IM (1997) Role of alpha 2-adrenoceptors in the regulation of intestinal water transport. *Br J Pharmacol* **120**:892-898.

Mansbach RS, Brooks EW, Sanner MA and Zorn SH (1998) Selective dopamine D4 receptor antagonists reverse apomorphine-induced blockade of prepulse inhibition. *Psychopharmacology (Berl)* **135**:194-200.

Mascia A, Afra J and Schoenen J (1998) Dopamine and migraine: a review of pharmacological, biochemical, neurophysiological, and therapeutic data. *Cephalalgia* **18**:174-182.

Millan MJ, Newman-Tancredi A, Audinot V, Cussac D, Lejeune F, Nicolas JP, Coge F, Galizzi JP, Boutin JA, Rivet JM, Dekeyne A and Gobert A (2000) Agonist and antagonist actions of yohimbine as compared to fluparoxan at alpha(2)-adrenergic receptors (AR)s, serotonin (5-HT)(1A), 5-HT(1B), 5-HT(1D) and dopamine D(2) and D(3) receptors. Significance for the modulation of frontocortical monoaminergic transmission and depressive states. *Synapse* **35**:79-95.

Millan MJ, Newman-Tancredi A, Quentric Y and Cussac D (2001) The "selective" dopamine D1 receptor antagonist, SCH23390, is a potent and high efficacy agonist at cloned human serotonin2C receptors. *Psychopharmacology (Berl)* **156**:58-62.

Missale C, Nash SR, Robinson SW, Jaber M and Caron MG (1998) Dopamine receptors: from structure to function. *Physiol Rev* **78**:189-225.

Olesen J, Diener HC, Husstedt IW, Goadsby PJ, Hall D, Meier U, Pollentier S and Lesko LM (2004) Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. *N Engl J Med* **350**:1104-1110.

Peroutka SJ (1997) Dopamine and migraine. *Neurology* **49**:650-656.

Peterfreund RA, Kosofsky BE and Fink JS (1995) Cellular localization of dopamine D2 receptor messenger RNA in the rat trigeminal ganglion. *Anesth Analg* **81**:1181-1185.

Polakowski JS, Segreti JA, Cox BF, Hsieh GC, Kolasa T, Moreland RB and Brioni JD (2004) Effects of selective dopamine receptor subtype agonists on cardiac contractility and regional haemodynamics in rats. *Clin Exp Pharmacol Physiol* **31**:837-841.

Ray BS and Wolff HG (1940) Experimental studies on headache. Pain sensitive structures of the head and their significance in headache. *Archives in Surgery* **41**:813-856.

Sinnott RS, Mach RH and Nader MA (1999) Dopamine D2/D3 receptors modulate cocaine's reinforcing and discriminative stimulus effects in rhesus monkeys. *Drug Alcohol Depend* **54**:97-110.

Undie AS and Friedman E (1988) Differences in the cataleptogenic actions of SCH23390 and selected classical neuroleptics. *Psychopharmacology (Berl)* **96**:311-316.

Villalon CM, Ramirez-San Juan E, Sanchez-Lopez A, Bravo G, Willems EW, Saxena PR and Centurion D (2003) Pharmacological profile of the vascular responses

to dopamine in the canine external carotid circulation. *Pharmacol Toxicol* **92**:165-172.

Waelkens J (1984) Dopamine blockade with domperidone: bridge between prophylactic and abortive treatment of migraine? A dose-finding study. *Cephalalgia* **4**:85-90.

Willems EW, Trion M, De Vries P, Heiligers JP, Villalon CM and Saxena PR (1999) Pharmacological evidence that alpha1-and alpha2-adrenoceptors mediate vasoconstriction of carotid arteriovenous anastomoses in anaesthetized pigs. *Br-J-Pharmacol.* **127**:1263-1271.

Williamson DJ, Hargreaves RJ, Hill RG and Shepheard SL (1997b) Sumatriptan inhibits neurogenic vasodilation of dural blood vessels in the anaesthetized rat--intravital microscope studies. *Cephalalgia* **17**:525-531.

Footnotes

The work has been supported by the Wellcome Trust.

Legends for figures

Figure 1. The effects of repeated electrical stimulation during dopamine infusion on dural blood vessel diameter. Following control responses to electrical stimulation rats were infused with dopamine A) 20 or 40 $\mu gkg^{-1}min^{-1}$ and electrical stimulation repeated during the infusion or B) infused with dopamine (20 $\mu gkg^{-1}min^{-1}$) after pre-treatment with either S(-)-eticlopride hydrochloride (3 $mgkg^{-1}$), R(+)-SCH-23390 (1.0 $mgkg^{-1}$) or yohimbine hydrochloride (3 $mgkg^{-1}$) and electrical stimulation repeated. * $P < 0.05$ significance compared to the control. # $P < 0.05$ significance compared to the NDV response with dopamine infusion.

Figure 2. The effects of repeated electrical stimulation during dopamine receptor agonist infusion on dural blood vessel diameter. Following control responses to electrical stimulation rats were infused with either A68930 hydrochloride or (-)-quinpirole hydrochloride (50 $\mu gkg^{-1}min^{-1}$) and electrical stimulation repeated during the infusion. In the case of A68930 hydrochloride, it was also pretreated with yohimbine (3 $mgkg^{-1}$) in a separate experiment. * $P < 0.05$ significance compared to the control. # $P < 0.05$ significance compared to the NDV response with A68930 hydrochloride infusion.

Figure 3. The effects of dopamine receptor antagonists on neurogenic dural vasodilation. Following control responses to electrical stimulation rats were treated with a dopamine receptor antagonist and electrical stimulation repeated. The highest dose used for each antagonist is represented.

Table 1 Summary of drugs used, sites of action and effective doses

Drug	Site(s) of action	Previously effective doses (ED_{50})	References
Dopamine hydrochloride	Endogenous dopamine agonist	Vasodilator = 0.4μM, Vasoconstrictor = 90μM Vasoconstrictor = 10 – 310 $\mu gkg^{-1}min^{-1}$	(Edvinsson et al., 1978a; Villalon et al., 2003)
A68930 hydrochloride	D_1-like receptor agonist	Rat caudate putamen = 2.5 nM Behavioural = 20 – 60 μgkg^{-1}	(Kebabian et al., 1990; DeNinno et al., 1991)
Quinpirole hydrochloride	D_2-like receptor agonist	Neocortex = 25nM Behavioural = 3 – 30 μgkg^{-1}	(Liebman et al., 1988; Sinnott et al., 1999)
R(+)-SCH-23390	D_1 receptor antagonist	Catalepsy = 0.1 $mgkg^{-1}$ Amphetamine block = 0.025 – 0.25 $mgkg^{-1}$	(Undie and Friedman, 1988; Bardo et al., 1999)
	5-HT_{2C} agonist	EC_{50} = 2.6 nM, Ki = 6.3nM	(Millan et al., 2001)
S(-)-eticlopride hydrochloride	D_2 receptor antagonist	Amphetamine block = 0.2 – 2 $mgkg^{-1}$	(Bardo et al., 1999)
U99194A maleate	D_3 receptor antagonist	Behavioural = 1.47 – 45 $mgkg^{-1}$ (s.c.)	(Clifford and Waddington, 1998)
L-745,870 hydrochloride	D_4 receptor antagonist	Neuroleptic = 1-10 $mgkg^{-1}$ (s.c.) Apomorphine inhibition = 0.1 – 3 $mgkg^{-1}$ (i.v.)	(Mansbach et al., 1998) (Kawashima et al., 1999)
Yohimbine hydrochloride	α_2-adrenoceptor and $D_{2/3}$ receptor antagonist, 5-$HT_{1A/1B/1D}$ receptor agonist	α_2-adrenoceptor inhibition – 2.5 $mgkg^{-1}$ (i.v.) UK 14,304 inhibition – 0.4-4 $mgkg^{-1}$ (i.v.)	(Hsu and Kakuk, 1984; Liu and Coupar, 1997) (Millan et al., 2000)

Table 2 Summary of the effects of dopamine receptor antagonists on neurogenic dural vasodilation (NDV)

Dopamine antagonist	Statistical significance*
D_1 antagonist – R(+)-SCH-23390 hyrdochloride (1 and 0.3 mgkg^{-1})	($F_{1,5}$ = 0.89, P = 0.39, n = 6)
D_2 antagonist – S(-)-eticlopride hydrochloride (0.3, 1 and 3 mgkg^{-1})	($F_{2,10}$ = 0.79, P = 0.91, n = 6)
D_3 antagonist – U99194A maleate (0.3, 1.0 and 3.0 mgkg^{-1})	($F_{2,10}$ = 0.16, P = 0.83, n = 6)
D_4 antagonist – L-745,870 hydrochloride (0.3, 1.0 and 3.0 mgkg^{-1})	($F_{2,10}$ = 0.66, P = 0.52, n = 5)

*ANOVA for repeated measures with dose as a within subject factor, with Bonferroni *post-hoc* correction for multiple comparisons. $P < 0.05$ statistical significance

Table 3 Summary of the effects of dopamine on dural blood vessel diameter and mean arterial blood pressure

Dosage ($\mu gkg^{-1}min^{-1}$)	**0.5**	**1**	**2**	**5**	**10**	**15**	**20**	**40**
Dural blood vessel change (%age)	↑ 3.12 ± 4.6	↑ 7.31 ± 2.5*	↓ 1.18 ± 1.8	↓ 3.14 ± 1.7	↓ 0.47 ± 2.0	↓ 5.21 ± 2.5	↓ 10.1 ± 3*	↓ 16.45 ± 4*
Blood pressure increase (mmHg)	1.43 ± 0.8	0.08 ± 1.5	0.19 ± 1.0	0.09 ± 1.0	3.34 ± 1.6	9.53 ± 2.8*	19.21 ± 3.1*	24.4 ± 3.6*

*$P < 0.05$ significant change compared to status prior to dopamine injection.
↑ increase, ↓ decrease.

Table 4 Summary of the effects of specific dopamine agonists on dural blood vessel diameter and arterial blood pressure

	A68930 hydrochloride				(-)-quinpirole hydrochloride			
Dosage of dopamine agonist ($\mu gkg^{-1}min^{-1}$)	**1**	**10**	**50**	**50 μgkg^{-1} and yohimbine (3 $mgkg^{-1}$)**	**1**	**10**	**50**	**50 μgkg^{-1} and yohimbine (3 $mgkg^{-1}$)**
Dural blood vessel change (%age)	↓ 2.58 ± 4	↓ 17.8 ± 4*	↓ 19.1 ± 6*	↓ 11.3 ± 2	↓ 4.6 ± 3	↓ 1.2 ± 3	↓ 0.86 ± 2	↑ 1.4 ± 4
Blood pressure change (mmHg)	↑ 4 ± 2	↑ 9.8 ± 4	↑ 31.9 ± 4*	↑ 10.7 ± 3*#	↓ 1.1 ± 1	↑ 0.1 ± 1	↑ 1.58 ± 1	↑ 2.2 ± 1

*$P < 0.05$ significant change compared to status prior to dopamine injection.
#$P < 0.05$ significant change when compared to change with just 50 μgkg^{-1} A68930
↑ increase, ↓ decrease.

Table 5 Summary of the dural blood vessel and blood pressure changes caused by the dopamine receptor antagonists

Dopamine antagonist	Dural blood vessel diameter change (%age)	Mean arterial blood pressure change (mmHg)
D_1 antagonist - R(+)-SCH-23390 (0.3 mgkg^{-1})	↓ 12.2 ± 3* (t_{10} = 3.49, P < 0.05)	↑ 10.2 ± 2* (t_{10} = 4.44, P < 0.05)
R(+)-SCH-23390 (1.0 mgkg^{-1})	↓ 33.4 ± 5* (t_{10} = 6.89, P < 0.05)	↑ 16.8 ± 4* (t_{10} = 4.76, P < 0.05)
D_2 antagonist - S(-)-eticlopride hydrochloride (0.3 mgkg^{-1})	↓ 2.3 ± 5 (t_{12} = 0.89, P = 0.389)	↓ 1.1 ± 5 (t_{12} = 0.236, P = 0.817)
S(-)-eticlopride hydrochloride (1.0 mgkg^{-1})	↑ 0.1 ± 8 (t_{12} = 0.757, P = 0.463)	↑ 6.3 ± 4 (t_{12} = 1.51, P = 0.157)
S(-)-eticlopride hydrochloride (3.0 mgkg^{-1})	↑ 9.7 ± 9 (t_{12} = 1.03, P = 0.324)	↑ 16.0 ± 5* (t_{12} = 3.038, P < 0.05)
D_3 antagonist - U99194A (0.3 mgkg^{-1})	↓ 1.6 ± 6 (t_{11} = 1.01, P = 0.295)	↑ 9.3 ± 3* (t_{11} = 3.34, P < 0.05)
U99194A (1.0 mgkg^{-1})	↓ 10.3 ± 3* (t_{11} = 2.98, P < 0.05)	↑ 8.4 ± 3* (t_{11} = 2.97, P < 0.05)
U99194A (3.0 mgkg^{-1})	↓ 10.7 ± 8 (t_{11} = 1.47, P = 0.17)	↑ 10.5 ± 3* (t_{11} = 3.21, P < 0.05)
D_4 antagonist - L-745,870 (0.3 mgkg^{-1})	↓ 0.8 ± 1 (t_{11} = 0.72, P = 0.49)	↓ 3.2 ± 3 (t_{11} = 1.08, P = 0.30)
L-745,870 (1.0 mgkg^{-1})	↓ 1.3 ± 0.4* (t_{11} = 3.12, P < 0.05)	↓ 3.0 ± 1* (t_{11} = 2.99, P < 0.05)
L-745,870 (3.0 mgkg^{-1})	↑ 1.0 ± 1 (t_{11} = 0.69, P = 0.5)	↓ 8.0 ± 2* (t_{11} = 3.43, P < 0.05)

*P < 0.05 significant change compared to status prior to dopamine antagonist injection

Figure 1A

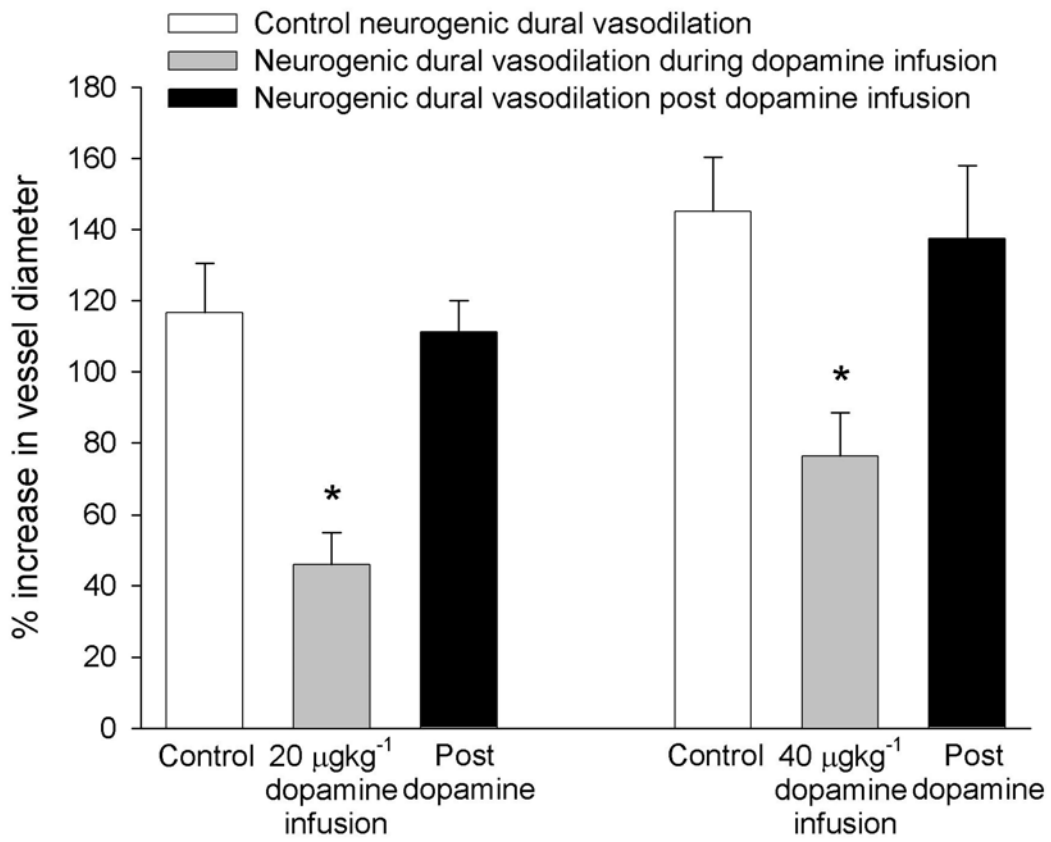

Figure 1B

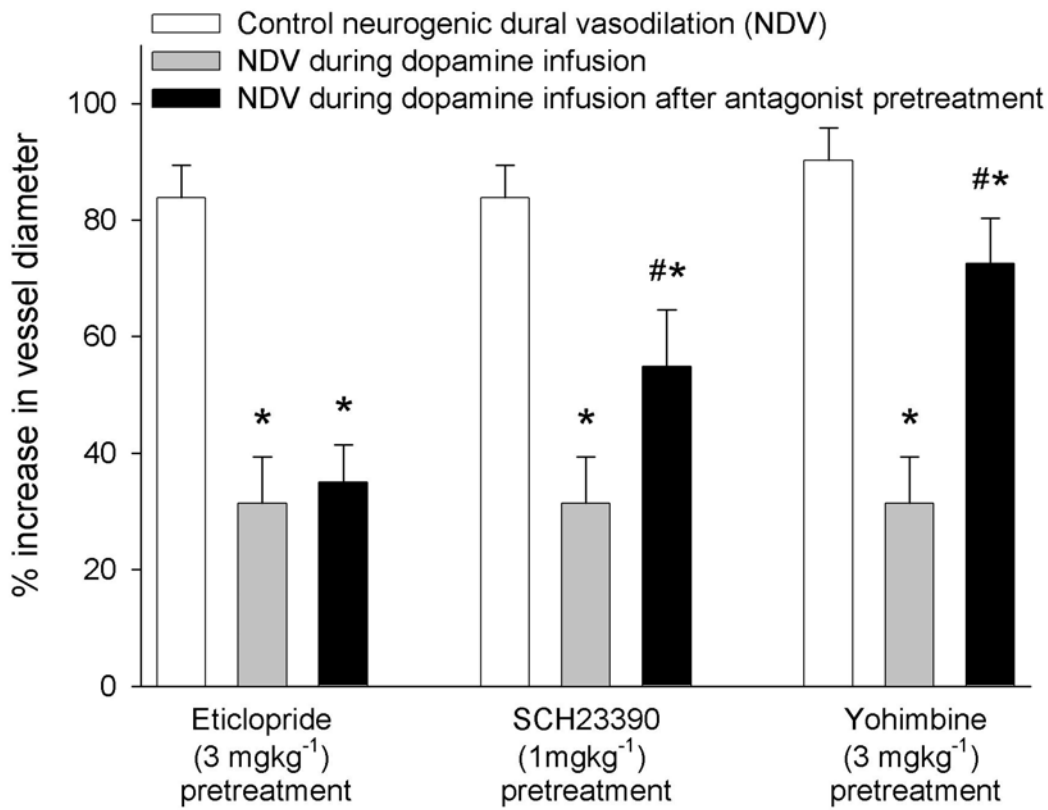

Figure 2

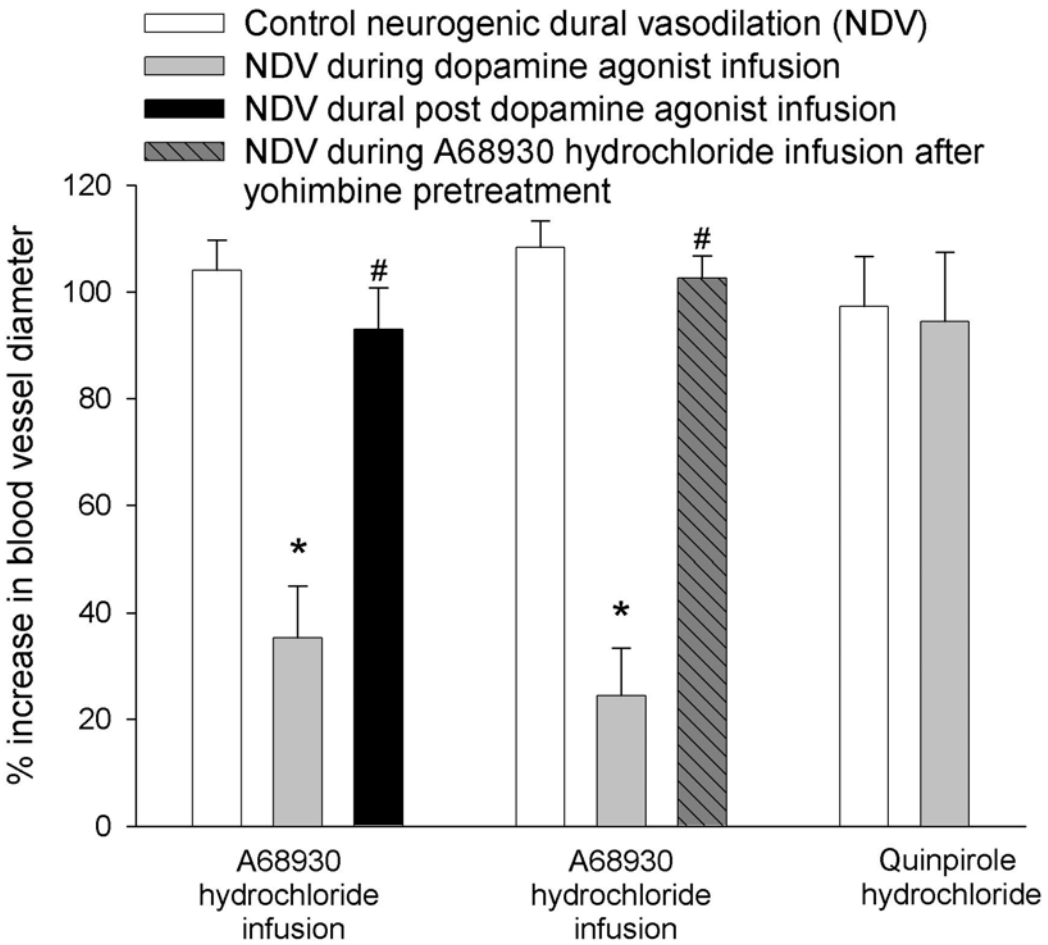

Figure 3