The Role of Dopamine in a Model of Trigeminovascular Nociception

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Received January 4, 2005; accepted March 16, 2005

ABSTRACT

Migraine is a common, disabling problem with three phases: premonitory, main headache attack, and postdrome. The headache phase is thought to involve activation of trigeminal neurons, whereas the premonitory and postdrome phases may involve dopaminergic mechanisms. In animal studies, dopamine has been found to cause vasodilatation of cranial arteries at very low doses. Using intravital microscopy, we examined the effect of dopamine receptor agonists on dural blood vessel caliber and the effect of dopamine and specific dopamine receptor antagonists on trigeminovascular neurogenic dural vasodilatation. Dopamine hydrochloride caused a significant vasodilatation (P < 0.05) and increase in arterial blood pressure (P < 0.05) that was reversed by a D2-receptor antagonist, yohimbine, rather than specific dopamine receptor antagonists. The D1 receptor agonist caused a vasoconstriction (P < 0.05) and a blood pressure increase (P < 0.05), which was reversed by yohimbine and therefore D2-receptor-mediated. None of the specific dopamine receptor antagonists were able to attenuate neurogenic dural vasodilatation. Dopamine hydrochloride infusion (P < 0.05) and a D1 receptor agonist were able to attenuate the vasodilatation (P < 0.05), with maximal dilation returning after cessation of the dopamine agonist infusion. This response may be due to the vasoconstrictor effects of the D2-receptor and an action at the D1 receptor. In the intravital model of trigeminal activation, it seems 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.

In addition to the headache phase in migraine, there are also the premonitory and resolution phases, which are characterized by nausea, vomiting, hypotension and drowsiness, and 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 D2-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 dopamine agonists caused slight vasodilation at very low doses, whereas at higher doses dopamine caused vasoconstriction (Edvinsson et al., 1978a,b). Similarly, dopamine and apomorphine intracon- rotid infusions caused a dose-dependent vasoconstriction in dog (Villalon et al., 2003), although they found that a selective D1 receptor agonist caused slight vasodilation, as did the effects of dopamine after it was antagonized by a α2-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 trigeminovas- cular activation uses the reaction of meningeal blood vessel caliber after electrical stimulation of a cranial window as a model of trigeminal nerve fiber activation (Williamson et al., 1997b), and it has proven to be an excellent model in predicting antimigraine efficacy (Williamson et al., 1997b). Previ- ously, 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 neuro- genic dural vasodilation. We also looked at the direct effects of dopamine and specific D1 and D2 receptor agonists on dural blood vessel caliber. The response of dopamine was also challenged with specific dopamine receptor antagonists as well as α2-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 performed under the UK Home Office (Scientific Procedures) Act (1986). Male Sprague-Dawley rats (280–385 g) were anesthetized throughout the experiments with sodium pen- tobarbitone (60 mg kg^{-1} i.p. initially and then with 18 mg kg^{-1} h^{-1} i.v. infusion). The left femoral artery and vein were cannulated for blood pressure recording and intravenous infusion of anesthetic, respectively. Temperature was maintained throughout using a homeothermic blanket system. The rats were placed in a stereotactic frame, the skull was exposed, and the right or left parietal bone was 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°C), and a branch of the middle meningeal artery was viewed using an intra- vital microscope (MV2100; Microvision, Runcorn, UK), and the im- age was displayed on a television monitor. Dural blood vessel diam- eter was continuously measure using a video dimension analyzer (Living Systems Instrumentation, Burlington, VT) and displayed with blood pressure on a data analysis system (Spike2 version 4; Cambridge Electronic Design, Cambridge, UK).

Experimental Protocols

Defining Electrical Stimulation Parameters. Electrical stim- ulation was used to evoke neurogenic dural vasodilation with a bipolar stimulating electrode (NE 200X; Clark Electromedical In- struments, Pangbourne, UK) that was placed on the surface of the cranial window approximately 200 μm from the vessel of interest. The surface of the cranial window was stimulated at 5 Hz, 1 ms for 10 s (Grass stimulator S88; Grass Instruments, Quincy, 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 An- tagonists on Neurogenic Dural Vasodilation. The effect of do- pamine hydrochloride and the specific dopamine receptor agonists A68930 hydrochloride and (-)-quinpirole hydrochloride on neuro- genic dural vasodilation were studied. Dopamine (20 or 40 μg kg^{-1} min^{-1}), A68930 hydrochloride (50 μg kg^{-1} min^{-1}), and (-)-quinpi- role hydrochloride (50 μg kg^{-1} min^{-1}) were intravenously infused at least 10 min after a control response to electrical stimulation. The electrical stimulation was then repeated 5 min into the infusion, and the infusion continued for a further 5 min. At least 10 min after the completion of the dopamine infusion, the electrical stimulation was repeated. The response of dopamine hydrochloride on neurogenic dural vasodilation was also challenged with the α2-adrenoceptor antagonist yohimbine (3 mg kg^{-1}).

In a separate series of experiments, the neurogenic dural vasodi- lator response was challenged with a D1 dopamine receptor antago- nist, R(+)-SCH-23390. A control response to electrical stimulation was followed at least 10 min later by an intravenous bolus of R(+)- SCH-23390 (0.5 mg kg^{-1}) and followed 5 min later by a repeat electrical stimulation. This protocol was repeated for an increased dose of R(+)-SCH-23390 (1.0 mg kg^{-1}) in the same animal. A simi- lar series of experiments were also completed with a D2 dopamine receptor antagonist, S(-)-eticlopride hydrochloride (0.3, 1.0, and 3.0 mg kg^{-1}); a D3 dopamine receptor antagonist, U99194A maleate (0.3, 1.0, and 3.0 mg kg^{-1}); and a D4 dopamine receptor antagonist, L-745,870 hydrochloride (0.3, 1.0, and 3 mg kg^{-1}).

Effects of Dopamine Receptor Agonists and Antagonists on Dural Blood Vessel Caliber. The effect of dopamine hydrochloride, the D1 receptor agonist A68930 hydrochloride, and the D2 receptor agonist (-)-quinpirole hydrochloride on dural blood vessel diameter was studied. Increasing doses of dopamine hydrochloride (0.5, 1, 2, 5, 10, 15, 20, and 40 μg kg^{-1} min^{-1}), A68930 hydrochloride (1, 10, and 50 μg kg^{-1} min^{-1}), and (-)-quinpirole (1, 10, and 50 μg kg^{-1} min^{-1}) were administered as an infusion for 10 min each, with a gap of 5 min between each increase of dose. In a separate series of experiments, the effects of dopamine hydrochloride were challenged with a D1 dopamine receptor antagonist, R(+)-SCH-23390. Dopamine was in- fused at 40 μg kg^{-1} min^{-1} for 10 min, and thereafter a further 10 min R(+)-SCH-23390 (0.3 mg kg^{-1}) was administered, and 5 min later a 10-min dopamine infusion was repeated. A further 10 min after the dopamine infusion was completed, an increased dosage of R(+)-SCH-23390 (1.0 mg kg^{-1}) was administered, and the dopa- mine infusion was repeated. A similar series of experiments was also completed with a D2 dopamine receptor antagonist, S(-)-eticlopride hydrochloride (0.3, 1.0, and 3.0 mg kg^{-1}); a D3 dopamine receptor antagonist, U99194A maleate (0.3, 1.0, and 3.0 mg kg^{-1}); and a D4 dopamine receptor antagonist, L-745,870 hydrochloride (0.3, 1.0, and 3 mg kg^{-1}).

In a separate series of experiments, we examined the response of 40 μg kg^{-1} min^{-1} dopamine hydrochloride infusion with yohimbine, the α2-adrenergic receptor antagonist. Using the protocol mentioned above, 40 μg kg^{-1} min^{-1} dopamine was infused for 10 min and was followed by yohimbine (3 mg kg^{-1}) that was followed 5 min later by a repeat of the dopamine infusion. A68930 hydrochloride (50 μg kg^{-1} min^{-1}) and (-)-quinpirole (50 μg kg^{-1} min^{-1}) were also both chal- lenged with yohimbine (3 mg kg^{-1}).

Data Analysis

The peak effects of electrical stimulation and dopamine infusion on dural vessel diameter was calculated as a percentage change from
the prestimulation baseline diameter. The nature of the experimental setup, where the magnification of the dural vessel selected for study was different in each setup, made it impractical to standardize the dural vessel measurement; therefore, the dural vessel diameter was measured in arbitrary units, and all calculations are related to the premanipulation baseline. The vessel size was approximately 150 to 200 μm. All data are expressed as mean ± S.E.M. Statistical analysis was performed using ANOVA for repeated measures with Bonferroni’s post hoc correction for multiple comparisons followed by Student’s paired t test where appropriate (SPSS version 10.0; SPSS Inc., Chicago, IL). 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 setup.

**Drugs**

Dopamine was purchased as dopamine hydrochloride predissolved in water ( Faulding Pharmaceuticals Plc, Warwick, UK). R- (+)-SCH-23390 hydrochloride, S- (–)-eticlopride hydrochloride [S- (–)-3-chloro-5-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-6-hydroxy-2-methoxybenzamide hydrochloride], U99194A maleate, and L-745,870 hydrochloride (each in a 10 mg ml⁻¹ solution and all from Sigma Chemical, Poole, Dorset, UK) were dissolved in water for injection as salts and administered in an approximate volume of 0.3 ml. A68930 hydrochloride and (–)-quinpirole hydrochloride [(4aRtrans)-4Aa,5,6,7,8Aa,9-octahydro-5-propyl-1H-pyrrozol][3,4-gquinoline] (Tocris Cookson Inc., Bristol, UK) were dissolved in water for injection. There is a summary of drugs used in Table 1.

**Results**

**Effects of Dopamine and Dopamine Receptor Antagonist of Neurogenic Dural Vasodilation.** Neurogenic dural vasodilation with electrical stimulation was significantly inhibited compared with control during infusion of 20 μg kg⁻¹ min⁻¹ dopamine, 116.7 ± 14 to 46 ± 9% (t₁₀ = 6.78, P < 0.05, n = 11); an uninhibited vasodilation was restored post-dopamine infusion, 46 ± 9 to 111.2 ± 8% (t₁₀ = −7.98, P < 0.05, n = 11). This is similarly the case for the 40 μg kg⁻¹ min⁻¹ dopamine infusion. Vasodilation was inhibited from 145.1 ± 15 to 76.3 ± 12% (t₅ = 3.92, P < 0.05, n = 7), and the full vasodilation was restored postdopamine infusion, 76.3 ± 12 to 137.5 ± 20% (t₆ = 2.93, P < 0.05, n = 7; Fig. 1A). Specific dopamine receptor agonists were also infused; A68930 hydrochloride (50 μg kg⁻¹ min⁻¹) was able to significantly inhibit neurogenic dural vasodilation from 102.0 ± 8 to 42.3 ± 14% (t₅ = 3.46, P < 0.05, n = 6), and the neurogenic dural vasodilation response returned post-A68930 hydrochloride infusion 42.3 ± 14 compared with 93.0 ± 14% (t₅ = −2.82, P < 0.05, n = 6). The neurogenic dural vasodilator response after (–)-quinpirole hydrochloride (50 μg kg⁻¹ min⁻¹) was not significant, 97.3 ± 9 compared with 94.5 ± 13% (t₅ = 0.43, P = 0.685, n = 6).

The dopamine hydrochloride-induced inhibition of neurogenic dural vasodilation was blocked by pretreatment with the α₂-adrenergic receptor antagonist yohimbine (3 mg kg⁻¹), 31.4 ± 8 compared with 72.5 ± 8% (t₅ = 2.26, P = 0.073, n = 6), and by the D₁ dopamine receptor antagonist R- (+)-SCH-23390 (1 mg kg⁻¹), 31.4 ± 8 compared with 54.8 ± 10% (t₅ = 4.4, P < 0.05, n = 6), although in each case the response was still significantly different. The dopamine hydrochloride response was not inhibited by the specific D₂ dopamine receptor antagonist S- (–)-etopride hydrochloride (3 mg kg⁻¹), 31.4 ± 8 compared with 35.0 ± 6% (t₅ = 9.99, P < 0.05, n = 6) (Fig. 1B). The A68930 hydrochloride-induced inhibition of neurogenic dural vasodilatation was also reversed by yohimbine (3 mg kg⁻¹), 24.49 ± 9% compared with 102.6 ± 4% (t₅ = −6.15, P < 0.05, n = 5) (Fig. 2).

None of the dopamine receptor antagonists were able to inhibit neurogenic dural vasodilation at the doses applied; data are summarized in Table 2 and Fig. 3. The effects of the dopamine antagonists themselves on dural blood vessel diameter and mean arterial blood pressure are summarized 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.

**Table 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Site(s) of Action</th>
<th>Previously Effective Doses (ED₅₀)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine hydrochloride</td>
<td>Endogenous dopamine agonist</td>
<td>Vasodilator = 0.4 μM; vasoconstrictor = 90 μM; vasoconstrictor = 10–310 μg kg⁻¹ min⁻¹</td>
<td>Edvinsson et al. (1978a); Villalon et al. (2003)</td>
</tr>
<tr>
<td>A68930 hydrochloride</td>
<td>D₁-like receptor agonist</td>
<td>Rat caudate putamen = 2.5 nM; behavioral = 20–60 μg kg⁻¹; Neocortex = 25 nM; behavioral = 3–30 μg kg⁻¹; Catelepsy = 0.1 mg kg⁻¹; amphetamine block = 0.025–0.25 mg kg⁻¹</td>
<td>Kebabian et al. (1990); DeNinno et al. (1991); Liebman et al. (1988); Sinnott et al. (1999); Undie and Friedman (1988); Bardo et al. (1999)</td>
</tr>
<tr>
<td>Quinpirole hydrochloride</td>
<td>D₂-like receptor agonist</td>
<td>NMDA = 2.6 nM; K = 6.3 nM</td>
<td>Millan et al. (2001)</td>
</tr>
<tr>
<td>R- (+)-SCH-23390</td>
<td>D₁ receptor antagonist</td>
<td>Amphetamine block = 0.2–2 mg kg⁻¹; Behavioral = 1.47–45 mg kg⁻¹ s.c.</td>
<td>Bardo et al. (1999); Clifford and Waddington (1998)</td>
</tr>
<tr>
<td>S- (–)-Eticlopride hydrochloride</td>
<td>D₄ receptor antagonist</td>
<td>Neuroleptic = 1–10 mg kg⁻¹ s.c.</td>
<td>Mansbach et al. (1998)</td>
</tr>
<tr>
<td>U99194A maleate</td>
<td>D₂ receptor antagonist</td>
<td>Apomorphine inhibition = 0.1–3 mg kg⁻¹ i.v.</td>
<td>Kawashima et al. (1999)</td>
</tr>
<tr>
<td>L-745,870 hydrochloride</td>
<td>D₃ receptor antagonist</td>
<td>α₂-Adrenergic receptor inhibition = 2.5 mg kg⁻¹ i.v.</td>
<td>Hsu and Kakuk (1984); Liu and Coupar (1997)</td>
</tr>
<tr>
<td>Yohimbine hydrochloride</td>
<td>α₂-Adrenergic receptor antagonist</td>
<td>5-HT₁A/A₁D receptor agonist</td>
<td>Millan et al. (2000)</td>
</tr>
</tbody>
</table>
blood pressure across all doses (Fig. 2). The slight change in dural blood vessel diameter was accompanied by a significant increase in arterial blood pressure in all animals tested (Table 3). The slight change in dural blood vessel diameter was accompanied by a significant increase in arterial blood pressure in all animals tested (Table 3).

### Effect of Dopamine Hydrochloride and D₁ and D₂ 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 μg kg⁻¹ min⁻¹), there was no significant effect on dural blood vessel diameter across all doses using an ANOVA for repeated measures (F[7,35] = 1.423, P = 0.288, n = 6), although using Student’s paired t test both the 20 (t₁₆ = 3.8, P < 0.05) and 40 μg kg⁻¹ min⁻¹ (t₃₉ = 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 arterial blood pressure across all doses (F[7,35] = 16.667, P < 0.0001, n = 6). The blood pressure change at the 15 μg kg⁻¹ min⁻¹ (t₁₅ = 3.427, P < 0.05), 20 μg kg⁻¹ min⁻¹ (t₁₆ = −6.15, P < 0.05), and 40 μg kg⁻¹ min⁻¹ (t₃₉ = −11.62, P < 0.05) doses were significant compared with the preinjection blood pressure (Table 3).

Dopamine hydrochloride (1, 10, and 50 μg kg⁻¹) did not cause any significant change in the dural blood vessel diameter across the cohort (F[2,10] = 2.3, P = 0.175, n = 6); however, using Student's paired t test, both 10 (t₅ = 4.5, P < 0.05) and 50 μg kg⁻¹ (t₁₃ = 3.85, P < 0.05) proved significant in all animals tested. There was a significant change in arterial blood pressure overall (F[2,10] = 27.6, P < 0.05, n = 6). The blood pressure change with the 50 μg kg⁻¹ dose of A68930 hydrochloride proved to be significant (t₁₃ = −7.88, P < 0.05). (−)-Quinpirole hydrochloride (1, 10, and 50 μg kg⁻¹) did not cause any significant change in both dural blood vessel diameter (F[2,10] = 0.381, P = 0.63, n = 6) or arterial blood pressure (F[2,10] = 2.78, P = 0.137, n = 6); these data are summarized in Table 4.

### Effects of Dopamine Receptor Antagonists and an α₂-Adrenergic Receptor Antagonist on Dural Blood Vessel Diameter and Mean Arterial Blood Pressure Changes Caused by Dopamine.

The effects of 40 μg kg⁻¹ min⁻¹ dopamine were challenged with various dopamine receptor...
antagonists. When the effects of 40 \( \mu g kg^{-1} min^{-1} \) dopamine on vessel diameter were compared with the preinjection diameter, there was a significant drop in diameter (\( t_{24} = 5.619, P < 0.001, n = 25 \)). \( R(-)+SCH-23930 \), 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 \( R(-)+SCH-23930 \) compared with the control response (\( F_{2,10} = 6.451, P = 0.029, n = 6 \)). With the 1.0 mg kg\(^{-1}\) dose of \( R(-)+SCH-23930 \), there was a 46.2 \% decrease with 34.1 \% mean arterial blood pressure (\( t_6 = 3.05, P = 0.028, n = 6 \)).

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

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

<table>
<thead>
<tr>
<th>Dosage (( \mu g kg^{-1} min^{-1} ))</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dural blood vessel change (percentage)</td>
<td>( 1.43 \pm 0.8 )</td>
<td>( 0.08 \pm 1.5 )</td>
<td>( 0.19 \pm 1.0 )</td>
<td>( 0.09 \pm 1.0 )</td>
<td>( 3.34 \pm 1.6 )</td>
<td>( 9.53 \pm 2.8^* )</td>
<td>( 19.21 \pm 3.1^* )</td>
<td>( 24.4 \pm 3.6^* )</td>
</tr>
<tr>
<td>Blood pressure increase (mm Hg)</td>
<td>( 7.31 \pm 2.5^* )</td>
<td>( 1.18 \pm 1.8 )</td>
<td>( 3.14 \pm 1.7 )</td>
<td>( 4.7 \pm 2.0 )</td>
<td>( 5.21 \pm 2.5 )</td>
<td>( 10.1 \pm 3^* )</td>
<td>( 16.45 \pm 4^* )</td>
<td>( 1.4 )</td>
</tr>
</tbody>
</table>

\( ^* \) increase; \( ^{1} \) decrease.

\( ^{1} P < 0.05 \) significant change compared with status before dopamine injection.

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

<table>
<thead>
<tr>
<th>Dosage of Dopamine Agonist</th>
<th>1</th>
<th>10</th>
<th>50 ( 50 \mu g kg^{-1} ) and Yohimbine (3 mg kg(^{-1}))</th>
<th>( (-)-quinpirole ) hydrochloride</th>
<th>1</th>
<th>10</th>
<th>50 ( 50 \mu g kg^{-1} ) and Yohimbine (3 mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dural blood vessel change (percentage)</td>
<td>( 2.58 \pm 4 )</td>
<td>( 17.8 \pm 4^* )</td>
<td>( 19.1 \pm 6^* )</td>
<td>( 11.3 \pm 2 )</td>
<td>( 4.6 \pm 3 )</td>
<td>( 1.2 \pm 3 )</td>
<td>( 0.88 \pm 2 )</td>
</tr>
<tr>
<td>Blood pressure change (mm Hg)</td>
<td>( 4 \pm 2 )</td>
<td>( 9.8 \pm 4 )</td>
<td>( 31.9 \pm 4^* )</td>
<td>( 10.7 \pm 3^* )</td>
<td>( 1.1 \pm 1 )</td>
<td>( 0.1 \pm 1 )</td>
<td>( 1.58 \pm 1 )</td>
</tr>
</tbody>
</table>

\( ^* \) increase; \( ^{1} \) decrease.

\( ^* P < 0.05 \) significant change compared with status before dopamine injection.

\( ^{1} P < 0.05 \) significant change compared with change with just 50 \( \mu g kg^{-1} \) A69890.
Dopamine and the Trigeminovascular System

Table 5

<table>
<thead>
<tr>
<th>Dopamine Antagonist</th>
<th>Dural Blood Vessel Diameter Change</th>
<th>Mean Arterial Blood Pressure Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mm Hg</td>
</tr>
<tr>
<td>D₂ antagonist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R(+)-SCH-23390 (0.3 mg kg⁻¹)</td>
<td>↓ 12.2 ± 3* (t₁₀ = 3.49, P &lt; 0.05)</td>
<td>↑ 10.2 ± 2* (t₁₀ = 4.44, P &lt; 0.05)</td>
</tr>
<tr>
<td>R(+)-SCH-23390 (1.0 mg kg⁻¹)</td>
<td>↓ 33.4 ± 5* (t₁₀ = 6.89, P &lt; 0.05)</td>
<td>↑ 16.8 ± 4* (t₁₀ = 4.76, P &lt; 0.05)</td>
</tr>
<tr>
<td>D₃ antagonist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S(-)-Eticlopride hydrochloride (0.3 mg kg⁻¹)</td>
<td>↑ 2.3 ± 5 (t₁₀ = 0.89, P = 0.389)</td>
<td>↓ 1.1 ± 5 (t₁₀ = 0.236, P = 0.817)</td>
</tr>
<tr>
<td>S(-)-Eticlopride hydrochloride (1.0 mg kg⁻¹)</td>
<td>↑ 0.1 ± 8 (t₁₀ = 0.757, P = 0.463)</td>
<td>↑ 6.3 ± 4 (t₁₀ = 1.51, P = 0.157)</td>
</tr>
<tr>
<td>S(-)-Eticlopride hydrochloride (3.0 mg kg⁻¹)</td>
<td>↑ 9.7 ± 9 (t₁₀ = 1.03, P = 0.324)</td>
<td>↑ 16.0 ± 5* (t₁₀ = 3.038, P &lt; 0.05)</td>
</tr>
<tr>
<td>D₄ antagonist</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U99194A (0.3 mg kg⁻¹)</td>
<td>↓ 1.6 ± 6 (t₁₁ = 1.01, P = 0.295)</td>
<td>↑ 9.3 ± 3* (t₁₁ = 3.34, P &lt; 0.05)</td>
</tr>
<tr>
<td>U99194A (1.0 mg kg⁻¹)</td>
<td>↓ 10.2 ± 3* (t₁₁ = 2.98, P &lt; 0.05)</td>
<td>↑ 8.4 ± 3* (t₁₁ = 2.97, P &lt; 0.05)</td>
</tr>
<tr>
<td>U99194A (3.0 mg kg⁻¹)</td>
<td>↓ 10.7 ± 8 (t₁₁ = 1.47, P = 0.17)</td>
<td>↑ 10.5 ± 3* (t₁₁ = 3.21, P &lt; 0.05)</td>
</tr>
<tr>
<td>L-745,870 (0.3 mg kg⁻¹)</td>
<td>↓ 0.8 ± 1 (t₁₁ = 0.72, P = 0.49)</td>
<td>↑ 3.2 ± 3 (t₁₁ = 1.08, P = 0.30)</td>
</tr>
<tr>
<td>L-745,870 (1.0 mg kg⁻¹)</td>
<td>↓ 1.3 ± 0.4* (t₁₁ = 3.12, P &lt; 0.05)</td>
<td>↑ 3.0 ± 1* (t₁₁ = 2.99, P &lt; 0.05)</td>
</tr>
<tr>
<td>L-745,870 (3.0 mg kg⁻¹)</td>
<td>↓ 1.0 ± 1 (t₁₁ = 0.69, P = 0.5)</td>
<td>↑ 8.0 ± 2* (t₁₁ = 3.43, P &lt; 0.05)</td>
</tr>
</tbody>
</table>

↑, increase; ↓, decrease.

* P < 0.05 significant change compared with status before dopamine antagonist injection.

Discussion

There are five different dopamine receptor subtypes thus far identified (D₁, D₂, D₃, D₄, and D₅), classified as D₁-like (D₁ and D₅), which are positively coupled to adenyl cyclase and D₂-like (D₂, D₃, and D₄), which are negatively coupled to adenyl 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 D₂ dopamine receptor antagonist, blocked nausea and vomiting caused by piribedil (Bes et al., 1986), we chose to antagonize dopamine receptors during neurogenic dural vasodilation. The D₁, D₂, D₃, and D₄ dopamine receptors were all unable to inhibit or attenuate neurogenic dural vasodilation.

By administering dopamine hydrochloride and A68930 hydrochloride, the D₁ 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 partially antagonized by the α₂-adrenoceptor antagonist yohimbine. The dopamine hydrochloride response was also partially attenuated by the D₁ receptor antagonist SCH-23390, although the antagonized response was still significantly less than the control neurogenic dural vasodilation. The D₃ receptor agonist had no effect on neurogenic dural vasodilation. The ability of dopamine hydrochloride to attenuate neurogenic 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 seem to be mediated by a noradrenergic response at the α₂-adrenoceptor (Edvinsson et al., 1978a, b; Willems et al., 1999; Villalon et al., 2003). It therefore seems that some of the inhibition is mediated by the α₂-adrenoceptor. It is possible that the D₁ dopamine receptor may contribute to this effect given the response of the agonist and antagonist in this model. The D₂ dopamine receptor antagonist was unable to fully inhibit the effects of the dopamine-induced inhibition; therefore, we describe a clear but partial response. The D₃ receptor agonist’s attenuation of neurogenic dural vasodilation was almost fully inhibited by α₂-adrenoceptor modulation; therefore, any action of dopamine agonists will be compromised by α₂-adrenoceptor activation and blood pressure effects. The partial D₃ dopamine receptor component in this model of trigeminovascular nociception may explain the actions of some migraine preventatives that are known to act on dopamine receptors (Peroutka, 1997; Mascia et al., 1998; Fanciullacci et al., 2000).

Neurogenic dural vasodilation is thought to result from the presynaptic 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 D₁ 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 α₂-adrenoceptors are manipulated (Akerman et al., 2001); therefore, it seems unlikely that dopamine is activating through prejunctional α₂-adrenoceptors to exert its inhibitory action in the trigeminovascular system. There is evidence that D₂ dopamine receptors are present in the trigeminal ganglion, using a cDNA probe and hybridization techniques (Peterfreund et al., 1995), although from the data they may not be transported to peripheral trigeminal nerve endings, so that antagonizing 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 $\alpha_2$-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 $\alpha_2$-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 $\alpha_2$-adrenoceptor is not involved in neurogenic dural vasoconstriction (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 $\alpha_2$-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 vasoconstriction. It was also found that $D_2$ receptor antagonists were unable to reverse the dopamine inhibition of neurogenic dural vasoconstriction, and given that $D_2$ receptors are considered $D_2$-like, we tentatively conclude that yohimbine effects are $\alpha_2$-adrenoceptor-specific.

As reported in pial, cerebral, and carotid arteries (Edvinsson et al., 1978a,b; Villalon et al., 2003), increasing doses of dopamine caused a vasoconstriction in the dural meningeal arteries. This was significant at the highest 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 $1 \mu g$ kg$^{-1}$ 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 vasoconstriction 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 $\alpha_2$-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 vasoconstriction 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 caliber and a significant increase in arterial blood pressure. These changes were reversed by an $\alpha_2$-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 $\alpha_2$-adrenoceptors rather than dopamine receptors.

Dopamine acts as a precursor to the catecholamines noradrenaline and adrenaline, which mediate vasoconstriction and blood pressure increase through the $\alpha_1$ and $\alpha_2$-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 mg kg$^{-1}$ 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 $\alpha_2$-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, wherein only vasoconstriction was inhibited by the $\alpha_2$-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 effects 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, whereas the $\alpha_2$-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 $\alpha_2$-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.

Acknowledgments

We thank Philip Holland, Kevin Shields, and Paul Hammond (Headache Group, Institute of Neurology) for assistance and technical support during these experiments.

References


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