Comparison of the effects of central and peripheral dopamine receptor activation on evoked firing in the trigeminocervical complex

Annabelle R Charbit, Simon Akerman and Peter J Goadsby

Headache Group (AC, SA, PJG)

Department of Neurology

University of California, San Francisco

San Francisco CA USA
Running title page

Running title: Dopamine and the trigeminocervical complex

Abbreviations: ANOVA, analysis of variance; MMA, middle meningeal artery; TCC, trigeminocervical complex; PSTH, post-stimulus histogram.

Chemical names: A68930 hydrochloride, cis-(±)-1-(Aminomethyl)-3,4-dihydro-3-phenyl-1H-2-benzopyran-5,6-diol hydrochloride; dihydrexidine hydrochloride, (±)-trans-10,11-Dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine hydrochloride; domperidone, 5-Chloro-1-[1-[3-(2,3-dihydro-2-oxo-1H-benzimidazol-1-yl)propyl]-4-piperidinyl]-1,3-dihydro-2H-benzimidazol-2-one; (-)-eticlopride hydrochloride, 3-Chloro-5-ethyl-N-[(2S)-1-ethyl-2-pyrrolidinyl]methyl]-6-hydroxy-2-methoxy-benzamide hydrochloride; fenoldopam hydrochloride, 6-Chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1H-3-benzazepine-7,8-diol hydrochloride; (-)-quinpirole hydrochloride, (4aR-trans)-4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride; remoxipride hydrochloride, (S)-(1-ethyl-2-pyrrolidinyl0methyl]2,6-dimethoxybenzamide hydrochloride

Correspondence:
Professor Peter J. Goadsby
Headache Group- Department of Neurology
University of California, San Francisco
505 Parnassus Avenue
San Francisco, CA 94143-0114
Tel: 415 353 8393
Abstract

Dopaminergic mechanisms have been suggested to play a role in migraine. Here electrophysiological techniques were used to study the effects of intravenously administered centrally or peripherally active dopamine receptor agonists and antagonists on evoked firing in the trigeminocervical complex (TCC). After establishing baseline firing evoked by electrical stimulation of the dural middle meningeal artery (MMA) and mechanical noxious and innocuous stimulation of the opthalmic dermatome, D₁ or D₂-like receptor agonists or antagonists were administered intravenously and the effect on firing determined. Additionally, using intravital microscopy, we monitored changes in dural vessel diameter in response to varying doses of D₁ or D₂-like receptor agonists to determine whether their effects were related to blood vessel caliber. The central D₂-like receptor agonist quinpirole hydrochloride inhibited firing in the TCC evoked by stimulation of the MMA. Conversely the central D₂-like receptor antagonists, eticlopride hydrochloride and remoxipride hydrochloride, facilitated MMA-evoked firing and also firing evoked by noxious and innocuous stimulation of the opthalmic dermatome. Both the peripheral D₁-like receptor agonist fenoldopam and the central D₁-like receptor agonists A68930 hydrochloride and dihydrexidine facilitated innocuous brush evoked firing, with A68930 hydrochloride having the greatest effect. The data suggest that dopamine binding to peripheral D₁-like receptors may play a role in peripheral sensitization, and that the inhibitory or excitatory effects seen with administration of dopamine receptor agonists are independent of blood vessel changes. Additionally these studies maintain that central D₂-like receptors inhibit trigeminocervical neurons, and may provide insight into the conflicting literature on the role of dopamine and its receptors in migraine.
Introduction

It is generally accepted that migraine involves activation, or the perception of activation, of trigeminovascular afferents (Goadsby et al., 2002). Among other substances putatively involved in migraine dopamine has certainly been implicated (Peroutka, 1997; Akerman and Goadsby, 2007). Recently, dopamine was found to attenuate nociceptive signaling when microiontophoresed directly into neurons in the trigeminocervical complex (TCC) activated by durovascular nociceptive stimulation (Bergerot et al., 2007). Furthermore, immunocytochemistry demonstrated that D₁ and D₂ dopamine receptors can be identified in the TCC (Bergerot et al., 2007). Additionally, the A11 dopaminergic nucleus, which provides the only known source of descending dopaminergic innervation for the spinal grey matter (Skagerberg et al., 1982), modulates trigeminal processing and this response is reversed by a D₂-like receptor antagonist (Charbit et al., 2006; Charbit et al., 2007). It has therefore been hypothesized that dopamine binds to inhibitory D₂-like receptors that exist post-synaptically on 2nd order neurons in the TCC, and thus inhibits the rostral transmission of nociceptive signals.

In contrast to this experimental data, there is evidence suggesting that dopamine blockade is useful in migraine. Most compelling is the fact that D₂-like receptor antagonists, such as prochlorperazine (Jones et al., 1996), metoclopramide (Colman et al., 2004), droperidol (Silberstein et al., 2003) and domperidone (Waelkens, 1984), have been used to treat migraine, although none has an exclusively dopaminergic pharmacology. It has been shown that stimulation of dural (Akerman and Goadsby, 2005), coronary, renal and mesenteric vascular D₂-like receptors causes indirect vasodilatation by inhibition of sympathetic vasoconstrictor tone.
(Lahlou, 1998; Amenta et al., 2002). Furthermore, stimulation of these vascular D₁-like receptors causes direct vasodilation with depression of blood pressure (Amenta et al., 2002). Dopamine may therefore be antinociceptive at central D₂ receptors in the TCC, and pro-nociceptive at peripheral D₁ and D₂-like receptors, or at other sites in the central nervous system.

The aim of this study was to compare, using electrophysiological techniques, the effects of intravenously administered central and peripheral dopamine receptor agonists and antagonists on evoked firing in the TCC. Additionally, using intravital microscopy, we looked at the response on dural blood vessel caliber of varying doses of quinpirole hydrochloride, fenoldopam and A68930 hydrochloride, to determine whether the observed changes of these D₂-like and D₁-like receptor agonists on dural vessel caliber contribute to their effects on trigeminovascular nociceptive processing.
Methods

Electrophysiology experiments

SURGERY: Fifty male Sprague Dawley rats (300-500g) were anesthetized throughout the experiments. Anesthesia was induced with intraperitoneal pentobarbital sodium (Sigma, UK), 60mg/kg⁻¹ in saline, and maintained by slow intravenous infusion of propofol at 40 mg/kg⁻¹/hr⁻¹ (Rapinovet®, Schering-Plough Animal Health). A sufficient depth of anesthesia was judged from the absence of withdrawal reflexes in the non-paralyzed state or from fluctuations in blood pressure during muscular paralysis. The left femoral artery and vein were exposed and cannulated for blood pressure recording and intravenous infusion of anesthetic, respectively. The rats were paralyzed with 1mg/kg pancuronium bromide (Pavulon™, Organon, Cambridge, UK) and ventilated with oxygen-enriched air, 2ml, 80-100 strokes per min (model 7025; Ugo Basile, Comerio, Italy). End-tidal CO₂ was monitored (Capstar 100, CWE Inc., USA) and maintained between 2.8-4.9%. Body temperature was monitored via a rectal thermometer and kept within physiological range at 36.9-37.9°C, using a homeothermic blanket system (Homeothermic blanket system for rodents, Harvard Instruments, UK). At the end of the experiment, animals were sacrificed with an overdose of anesthetic (pentobarbital, 0.7mL of 200mg / mL). The rats were placed in a stereotaxic frame (Model 1600 stereotaxic frame, David Kopf Instruments, USA), the skull was exposed and a craniotomy of the left parietal bone was performed with a saline-cooled drill (MF-Perfecta with 945 hand piece, W&H), to expose the middle meningeal artery (MMA), which was then bathed in mineral oil, to prevent dehydration. The skin and muscles of the dorsal neck were incised and separated, and a full C₁ laminectomy was carried out. The underlying dura mater was incised and removed to expose the dorsal spinal
cord. All experiments were conducted under a project and personal license issued by the UK Home Office under the Animals (Scientific Procedures) Act 1986, or in accordance with guidelines of the University of California San Francisco, Institutional Animal Care and Use Committee.

**STIMULATION OF THE MMA AND RECORDING FROM THE TCC:** A bipolar stimulating electrode (NE-200, Harvard Apparatus, UK) was placed directly onto the dura mater, adjacent to or straddling the MMA, and square-wave stimuli (0.5 Hz) of 0.5 ms duration and 8-15 V was applied (Grass S88, Grass Instrumentation, USA). Extracellular recordings were made in the ipsilateral trigeminocervical complex (TCC) from neurons receiving inputs from neurons with Aδ range latency (5-20 ms), responding to both electrical stimulation of the middle meningeal artery and mechanical noxious and innocuous stimulation of the facial receptive field, using a tungsten microelectrode of impedance 0.5-2 MΩ and tip diameter of 0.5 μm (tungsten metal microelectrode, Harvard Apparatus, UK). The recording electrode, suspended on a piezoelectric microdrive (EXFO, Burleigh Products Inc, USA) attached to the stereotaxic frame, was driven by a controller (EXFO Controller 8200, Inchworm, USA). As the electrode was lowered, neurons encountered were tested for convergent input from the cutaneous receptive field, using noxious pinch and innocuous brush, and from the dura mater, using electrical stimulation of the middle meningeal artery/dura mater (8-16 V, 0.5 ms, 0.5 Hz for 20 sweeps). Signal from the recording electrode, was fed through an AC preamplifier (Neurolog NL104, gain x2000, Digitimer, UK), through filters (Neurolog NL125; bandwidth 300 Hz to 20 kHz) and a 50 Hz noise eliminator (Humbug, Quest Scientific, Canada), and through a second stage amplifier (Neurolog NL106, gain x200-x999). The filtered and amplified electrical signal was routed to a
loudspeaker, via a power amplifier (Neurolog NL120), and was displayed on analogue and
digital-storage oscilloscopes (Goldstar, LG Precision, Korea; Metrix Electronics, France), which
displayed a single voltage pulse (or spike) of given height and duration in response to each action
potential whose height fell within a pre-set window of a window discriminator. The window
discriminator output data was then displayed on a computer as either a peri-stimulus histogram
or a post stimulus histogram (PSTH). The signal was also fed to an analogue to digital converter
(Cambridge Electronic Design, Cambridge, UK) and then to a personal computer.

EXPERIMENTAL PROTOCOLS: The first step in all the experiments was to achieve three consistent,
consecutive baseline responses to trigeminal nerve firing evoked by stimulation of the middle
meningeal artery and facial receptive fields. Then all stimulation and recording parameters
remained the same throughout the experiment. Peri-stimulus histograms were used for observing
the evoked firing in response to noxious and innocuous cutaneous stimulations (pinch and
brush), and post-stimulus histograms were used for viewing units that fired in response to
electrical stimulation of the MMA. Extracellular recordings were made in the TCC, from cells
with inputs whose fibers were activated consistent with Aδ latency, responding to both electrical
stimulation of the MMA and to mechanical noxious and innocuous stimulation of the ipsilateral
ophthalmic territory of the face.

DRUGS: All drugs were dissolved in water and, where necessary, the pH was adjusted to 7.0
using either a drop of HCl or NaHCO₃. Table 1 describes the D₁ and D₂ like receptor agonists
and antagonists that were administered. Some of these drugs did not cross the blood-brain
barrier, and were therefore considered peripheral specific receptor agonists or antagonists. The
drugs were made fresh on the morning of the experiment. Following baseline recordings, drugs were administered via the cannulated femoral vein, at volumes of 0.4 - 0.8 mL, and the effect of each drug on baseline evoked firing was monitored at five minute intervals for forty minutes.

DATA ANALYSIS: At the start of every experiment, three consistent consecutive baseline recordings of trigeminal firing evoked by stimulation of the MMA and receptive fields (noxious pinch and innocuous brush) were collected. All subsequent data was expressed as a percentage of the mean of those baselines. Data were assessed using an analysis of variance for repeated measures (ANOVA) test. If this was statistically significant, the data was also grouped into individual time points and analyzed using a Student’s paired t-test with Bonferroni post-hoc correction for multiple comparisons. Summary data are expressed as the mean ± SEM. Significance was assessed at the $P < 0.05$ level.

POST SURGICAL EXAMINATION OF TISSUE: At the end of the experiment and just prior to terminal anesthesia, a lesion was made in the TCC, by passing a current down the recording electrode (20 μA, 50 Hz, 0.5 ms for 120 secs). Following terminal anesthesia, the brain was removed and coronal sections 60 μm thick were cut and visualized under the light microscope (Zeiss, Axioplan Microscope), using the rat brain atlas (Paxinos and Watson, 2005) for reference. Recovered lesions revealed the locations of the recording electrode in the TCC and other sites could be reconstructed from microdrive readings.
Intravital microscopy experiments

We wanted to determine whether the observed effects of dopaminergic compounds in the trigeminocervical complex were related to dural blood vessel changes, particularly for D1-like and D2-like receptor agonists. We performed a separate set of experiments, in which rats were given varying intravenous doses of the centrally acting D2-like receptor agonist quinpirole hydrochloride, the peripheral D1-like receptor agonist fenoldopam hdyrochloride, and the centrally acting selective and full D1-like receptor agonists A68930 hydrochloride and dihydrexidine, and the effects on dural blood vessel diameter, as well as blood pressure, were monitored. For each drug, two or three smaller doses were given first, and finally the dose matching that given in the electrophysiological study above.

SURGERY: Experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee (IUCAC) at the University of California, San Francisco. All animals (n = 14) were anesthetized with pentobarbital (NembutalR 80 mg/ml, initially 60 mg/kg and maintained on an infusion at 40 mg/kg/hr. In order to visualize and measure the diameter of the middle meningeal artery, this artery was exposed, and the bone left intact: the surgery on the skull is similar to the electrophysiological set-up, except drilling was used to create a thinned cranial window, approximately 5x5 mm, in the parietal bone, until the middle meningeal artery was clearly visible under the bone, as described previously (Akerman and Goadsby, 2005). Care was taken to avoid damage to the dura mater by application of cooling saline to prevent overheating. A zoom lens (80-450x magnification), connected to an intravital microscope (MS-500C microscopeman, Moritex, UK) was positioned above the cranial window and the image of the dural artery displayed on a standard television monitor. Dural blood vessel diameter was
continually measured using a video dimension analyzer (Living Systems Instrumentation, USA).

For these intravital microscopy experiments, no further surgery was required. The animal was left for up to one hour before beginning experimentation, so that blood vessel diameter, which dilates during drilling, could return to its resting size.

**EXPERIMENTAL PROTOCOL AND DRUGS:** The baseline blood vessel diameter and blood pressure was observed for five minutes, followed by intravenous administration of the drug. When blood vessel diameter and blood pressure returned to normal, and no less than five minutes post injection, the next drug dose was given. The peripheral D<sub>1</sub>-like receptor agonist, fenoldopam hydrochloride was administered intravenously in doses of 3 µg/kg, 10 µg/kg, 30 µg/kg and 1mg/kg, and, and the effect of the drugs on dural blood vessel caliber was observed. The centrally acting D<sub>2</sub>-like receptor agonist, quinpirole hydrochloride, was administered intravenously in doses of 50 µg/kg, 150 µg/kg, 300 µg/kg and 3 mg/kg. The centrally acting D<sub>1</sub>-like receptor agonist, A68930 hydrochloride was administered intravenously in doses of 100 µg/kg, 300 µg/kg and 1 mg/kg, and dihydrexidine hydrochloride in doses of 0.5 mg/kg, 1 mg/kg and 3 mg/kg.

**DATA ANALYSIS:** The nature of the experimental setup, where the magnification of the dural vessel visualized was different in each animal by virtue of selecting an appropriate target vessel, made it impossible to standardise the dural vessel measurement; therefore, the change in dural vessel diameter is reported as a percentage change from pre-stimulation diameter (baseline maximal stimulation response = 100%). The vessels were typically in the range of 120-150 µm diameter. Summary data, mean ± SEM, are therefore expressed as a percentage of the mean of
the baseline diameter. Data were analyzed using a Student’s paired t-test. Significance was assessed at the \( P < 0.05 \) level.
Results

Electrophysiological data

Extracellular recordings in the trigeminocervical complex (TCC) were made from a total of fifty wide-dynamic range neurons, responsive to dural/middle meningeal artery (MMA) stimulation (8-15 V, 0.5 ms, 0.6 Hz), and with cutaneous receptive fields in the ophthalmic division of the trigeminal nerve (Figure 1, A). Neurons responding to MMA stimulation responded at latencies of 6-20 ms, hence were receiving input from Aδ fibers (Figure 1, B). The location of these neurons ranged from superficial to deep layers (laminae I to V) of the dorsal horn of the TCC, at depths ranging from 135-1106 μm (Figure 1, C).

Effect of intravenous D₂-like receptor specific drugs on evoked firing

D₂-like receptor agonist- central: Upon administration of the central D₂-like receptor agonist, quinpirole hydrochloride (2 mg/kg; n = 7), there was an immediate drop in blood pressure by 20-30 mmHg, that fully recovered after 60 seconds (Figure 2). MMA-evoked firing was significantly decreased by 17 ± 3% (F₂,5,15.0 = 2.47; P < 0.05; n = 7) below baseline over 40 minutes. The greatest point of inhibition was at five minutes, by 25 ± 9% (t₆ = 2.94; P < 0.05; n = 7) below baseline firing. Noxious pinch and innocuous brush-evoked firing remained around baseline at all time points from 5 to 40 minutes, with no significance across the cohort (Figure 3, A; 5, A).

D₂-like receptor antagonist- central: Following administration of the central D₂-like receptor antagonists, S-(-)-eticlopride hydrochloride (3 mg/kg; n = 5) or remoxipride hydrochloride (8
mg/kg; n = 7), there was a transient drop in blood pressure by 15-35 mmHg that fully recovered after 12-14 seconds for eticlopride hydrochloride and 45-50 seconds for remoxipride hydrochloride (Figure 2). With eticlopride hydrochloride, MMA-evoked firing significantly increased by 14 ± 3% ($F_{5,30} = 2.73; P < 0.05; n = 7$) above baseline over 25 minutes, with maximal facilitation at five minutes by 17 ± 5% ($t_6 = -3.74; P < 0.05; n = 7$) above baseline firing. Noxious pinch-evoked firing showed a trend for facilitation, though this was not significant over any time period, and innocuous brush-evoked firing remained around baseline throughout (Figure 3, B; 5, B). With remoxipride hydrochloride, MMA-evoked firing significantly increased by 20 ± 4% ($F_{3,15} = 6.84; P < 0.05; n = 7$) above baseline over 15 minutes, with maximal facilitation at 10 minutes by 32 ± 4% ($t_6 = -7.43; P < 0.05; n = 7$). Noxious pinch-evoked firing significantly increased by 28 ± 6% ($F_{4,24} = 2.89; P < 0.05; n = 7$), above baseline over 20 minutes with maximal facilitation at 5 minutes by 46 ± 18% ($t_6 = -2.54; P < 0.05; n = 7$). Innocuous brush-evoked firing significantly increased by 24 ± 3% ($F_{5,30} = 2.92; P < 0.05; n = 7$) above baseline over 25 minutes, with maximal facilitation at 25 minutes by 29 ± 6% ($t_6 = -4.92; P < 0.05; n = 7$). (Figure 3, C; 5, C).

**D$_2$-like receptor antagonist- peripheral:** The peripheral D$_2$-like receptor antagonist, domperidone (1.5 mg/kg; n = 5), had no effect on blood pressure (Figure 2), and MMA, noxious pinch and innocuous brush-evoked firing remained around baseline at all time points from 5 to 40 minutes, with no significance across the cohort (Figure 3, D).

**D$_2$-like receptor agonist central & D$_2$-like receptor antagonist- peripheral:** Following administration of the central D$_2$-like receptor agonist, quinpirole hydrochloride (3 mg/kg) plus
the peripheral D₂-like receptor antagonist, domperidone (1.5 mg/kg; n = 7), there was an immediate drop in blood pressure by 20-30 mmHg, that lasted no more than 60 seconds (Figure 2). MMA-evoked firing significantly decreased by 13 ± 2% \((F_{3,18.3} = 2.63; P < 0.05; n = 7)\) below baseline over a 40 minute cohort. The greatest point of inhibition was at 5 minutes, by 21 ± 5% \((t_6 = 3.89; *P < 0.05; n = 7)\) below baseline firing. Noxious pinch and innocuous brush-evoked firing remained around baseline at all time points, with no significance across the cohort (Figure 3, E; 5, D).

**Effect of intravenous D₁-like receptor specific drugs on evoked firing**

Following administration of the peripheral D₁-like receptor agonist fenoldopam hydrochloride (1 mg/kg; n = 8), there was an immediate drop in blood pressure by 30-40 mmHg, that fully recovered after 65-125 seconds (Figure 2). Innocuous brush-evoked firing significantly increased by 20 ± 3% \((F_{2.9,20.5} = 2.63; P < 0.05; n = 8)\) above baseline firing over a 40 minute cohort. MMA and noxious pinch-evoked firing remained around baseline at all time points from 5 to 40 minutes, with no significance across the cohort (Figure 4, A; 6, A).

Upon administration of the centrally active, full and irreversible D₁-like receptor agonist, A68930 hydrochloride (1 mg/kg; n = 8) there was an immediate rise in blood pressure by 30-60 mmHg, that lasted for 75-175 seconds, though it only fully recovered after about 10 minutes (Figure 2). Contrary to this, administration of the centrally active, full and reversible D₁-like receptor agonist, dihydrexidine hydrochloride (3 mg/kg, n = 8) produced an instant decline in blood pressure by 28-36 mmHg, that lasted for 12-18 minutes (Figure 2). Both A68930 hydrochloride and dihydrexidine had a facilitatory effect on innocuous brush-evoked firing.
A68930 hydrochloride caused innocuous brush-evoked firing to significantly increase by 36 ± 4% ($F_{7.49} = 2.29; P < 0.05; n = 8$) above baseline across a 35 minute cohort, starting at 10 minutes post injection. MMA-evoked firing remained around baseline at all time points from 5 to 40 minutes, whilst noxious pinch-evoked firing showed a trend for facilitation, though this was not significant over any time period (Figure 4, B; 6, B). Dihydrexidine hydrochloride caused innocuous brush-evoked firing to significantly increase by 20 ± 4% ($F_{7.49} = 3.39; P < 0.05; n = 8$) above baseline, and noxious pinch-evoked firing to significantly increase by 23 ± 3% ($F_{7.49} = 2.34; P < 0.05; n = 8$) above baseline, both across a 35 minute cohort, starting at 5 minutes post injection (Figure 4, C; 6, C).

The central D$_1$-like receptor antagonist, SCH 23390 (1 mg/kg; n = 5) had no effect on blood pressure (Figure 2), and MMA, noxious pinch and innocuous brush-evoked firing remained around baseline at all time points from 5 to 40 minutes, with no significance across the cohort (Figure 4, D).

The intravenous water control did not significantly affect MMA, noxious pinch or innocuous brush-evoked firing in the TCC from 5 to 40 minutes (Figure 4, E).

*Intravital microscopy*

The centrally acting D$_2$-like receptor agonist, quinpirole hydrochloride produced a dose-dependent increase in blood vessel diameter at all doses, 50 µg/kg, 150 µg/kg, 300 µg/kg and 1 mg/kg. Mean dilations were 31 ± 9 % ($t_4 = -3.69; *P < 0.05; n = 5$), 55 ± 6 % ($t_4 = -9.41; *P < 0.05; n = 5$), 68 ± 9 % ($t_4 = -8.08; *P < 0.05; n = 5$) and 83 ± 10 % ($t_4 = -8.79; *P < 0.05; n = 5$)
above the baseline reading, respectively. This was accompanied by a decrease in arterial blood pressure, as described above (Figure 7, A).

The peripheral D₁-like receptor agonist, fenoldopam hydrochloride, produced a significant increase in blood vessel diameter at 30 µg / kg and at 1 mg / kg, by 59 ± 10 % \((t_4 = -5.75; \*P < 0.05; n = 5)\) and 54 ± 12 % \((t_4 = -4.74; \*P < 0.05; n = 5)\) higher than the baseline reading, respectively. This was accompanied by the aforementioned decrease in arterial blood pressure (Figure 7, B).

The centrally acting D₁-like receptor agonist, A68930 hydrochloride, produced a significant dose-dependent decrease in blood vessel diameter at all doses, 100 µg/kg, 300 µg/kg and 1 mg/kg. The mean constrictions were 39 ± 10 % \((t_4 = 4.14; \*P < 0.05; n = 5)\), 33 ± 6 % \((t_4 = 5.43; \*P < 0.05; n = 5)\) and 31 ± 11 % \((t_4 = 2.96; \*P < 0.05; n = 5)\) lower than the baseline reading, respectively. This was accompanied by a decrease in arterial blood pressure at the lowest dose of 100 µg / kg, and then by the aforementioned increase in blood pressure at the higher doses (Figure 7, C).

The centrally acting D₁-like receptor agonist, dihydrexidine hydrochloride, also produced a significant dose-dependent decrease in blood vessel diameter at all doses, 0.5 mg/kg, 1 mg/kg and 3 mg/kg. Mean constrictions were 14 ± 4 % \((t_4 = 3.91; \*P < 0.05; n = 5)\), 32 ± 5 % \((t_4 = 7.44; \*P < 0.05; n = 5)\) and 43 ± 7 % \((t_4 = 6.25; \*P < 0.05; n = 5)\) below baseline, respectively. This was accompanied by a decrease in arterial blood pressure, as described earlier (Figure 7, D).
Discussion

The data demonstrate that dopamine D2 receptor agonists that cross the blood-brain barrier inhibit neurons in the trigeminocervical complex (TCC) with dural inputs and D2 receptor antagonists facilitate these neurons. In contrast peripherally restricted D2 receptor antagonists have no effect on TCC responses alone and do not alter the effect of centrally active D2 receptor agonists. D1 receptor agonists affect cutaneous nociceptive and non-nociceptive inputs to the TCC. Taken together the data are consistent with a predominantly central effect of dopamine on TCC neurons, with a potential role for peripheral D1 receptors in sensitization.

D2-like receptor effects

The D2-like receptor agonist, quinpirole and quinpirole + peripheral D2-like receptor antagonist, domperidone had a similar degree of inhibition of MMA-evoked firing. Quinpirole hydrochloride crosses the blood brain barrier, and its effect is consistent with the finding that dopamine has antinociceptive properties at D2-like receptors located centrally in the TCC (Bergerot et al., 2007). It is interesting to note that quinpirole was shown to cause a dose-dependent transient vasodilation that lasted as long as the blood pressure drop, yet there was no evidence of neuronal facilitation during this time period, neither from spontaneous activity nor evoked firing. With quinpirole still effective at inhibiting nociceptive evoked firing in the TCC and no evidence of a facilitatory effect, the data contribute to the argument that dural blood vessel dilation is not a sufficient determinant of trigeminocervical neuronal activity.
Both eticlopride and remoxipride, D₂-like receptor antagonists that cross the blood brain barrier, significantly facilitated MMA-evoked firing in the TCC, and in the case of remoxipride hydrochloride, noxious pinch and innocuous brush-evoked firing was also significantly facilitated. Eticlopride and remoxipride, both substituted benzamides, are atypical antipsychotics (Hogberg et al., 1987). Ideally we would have liked to use a typical D₂-like receptor antagonist such as haloperidol, but did not find one that was easy to dissolve. Indeed we attempted to use haloperidol, but found its dissolution parameters to be problematic for intravenous use. The solvent required dimethyl sulfoxide at all dilutions we tested resulted in a substantial drop in blood pressure and loss of neuronal activity. It is important to note that these substituted benzamides do show selective and potent affinity for the D₂-like receptor (Hogberg et al., 1987). Remoxipride, for instance, has been found to be considerably (50-fold) more potent than sulpiride in antagonising the effects of apomorphine in the rat (Nadal, 2001). The facilitatory effects of eticlopride and remoxipride on evoked firing in the TCC suggest the existence of a tonic dopaminergic inhibition of neuronal firing in the TCC in response to nociceptive stimuli, which is abolished when the D₂-like receptors are blocked.

Domperidone alone had no effect on evoked firing in the TCC, suggesting that peripheral D₂-like receptors do not play a role in trigeminal nerve activation. Domperidone, however has been shown to prevent the occurrence of migraine when taken during the premonitory phase of the attack (Waelkens, 1984). Peripheral D₂-like receptors are located presynaptically on sympathetic nerves, where they inhibit the release of norepinephrine (Kohli et al., 1983), suggesting that D₂ receptors are indirect vasodilators by inhibition of vasoconstriction (Kohli et al., 1983; Lahlou, 1998; Amenta et al., 2002). Additionally D₂-like receptors, both peripheral (e.g. causing
hypotension and gastrokinetic changes) and central (e.g. causing yawning, nausea and vomiting),
may have a role in the expression of the premonitory symptoms of migraine since blockade of
D2-like receptors has been shown to relieve these premonitory symptoms (Waelkens, 1984). In
contrast, D2-like receptor antagonists of varying specificity have been shown to be effective in
migraine (Waelkens, 1984; Silberstein et al., 2003). The question is where might this pain
relieving action take place, if not in the trigeminocervical complex?

**D1-like receptor effects**

Both the centrally active (A68930 & dihydrexidine) and the peripheral only (fenoldopam) D1-
like receptor agonists significantly facilitated innocuous brush-evoked firing in the TCC.
Dihydrexidine also facilitated noxious pinch-evoked firing. Fenoldopam is a selective agonist at
D1-like dopamine receptors and binds with moderate antagonist affinity to α2-adrenoceptors
(Ohlstein et al., 1985). It has no significant affinity for D2-like receptors, α1 and β-adrenoceptors,
5HT1 and 5HT2 receptors, or muscarinic receptors. In radiolabeled studies in rats, no more than
0.005% of fenoldopam crossed the blood-brain barrier, hence it is a peripheral D1-like receptor
agonist (Flaim et al., 1985). A68930 is a selective, full efficacy, irreversible D1-like dopamine
receptor agonist (DeNinno et al., 1990; Grenader and Healy, 1992), that binds very weakly to
D2-like receptors, and is virtually inactive at α- and β- adrenoreceptors. It readily crosses the
blood-brain barrier, and so is considered a central D1-like receptor agonist (Kebabian et al.,
1990). Dihydrexidine is a selective, full efficacy and reversible D1-like dopamine receptor
agonist, with no agonist activity at peripheral D2 receptors or adrenoceptors. dihydrexidine
appears to be fully bioavailable in brain and exhibits profound anti-parkinsonism effects in vivo
(Brewster et al., 1990). The fact that both the centrally active and the peripheral only D1-like
receptor agonists facilitated innocuous brush-evoked firing, suggests that the facilitatory action is at least at peripheral D1-like receptors. The effect of A68930 was greater than that of both fenoldopam and dihydrexidine, which were equal in their overall facilitation of innocuous brush-evoked firing. A68930 has been calculated to be twelve times more potent than fenoldopam (Christie and Smith, 1994), and about thirteen times more potent than dihydrexidine (Watts et al., 1995). However, the high doses used in these experiments most likely saturated the D1 receptor. Therefore the observed differences in the degree of facilitation after intravenous administration of these D1 receptor agonists was probably due, not to potency of the drugs, but to some other feature such as off-target action, functional selectivity at D1 signalling systems, or differential functional actions at D1 versus D5 receptors.

D1-like receptors are located on vascular smooth muscles and subserve vasodilation of cerebral, coronary, renal and mesenteric blood vessels (Amenta et al., 2002), and fenoldopam is a rapid-acting peripheral vasodilator. A68930 hydrochloride, however has been shown to be a rapid-acting vasoconstrictor of the middle meningeal artery when administered intravenously (Akerman and Goadsby, 2005). Indeed we demonstrated this here using intravital microscopy, and it is interesting to note that whilst both A68930 hydrochloride and dihydrexidine had a constrictive effect on blood vessel caliber, and fenoldopam a dilatory effect, all three drugs caused sensitization. This suggests that facilitation in the TCC is independent of blood vessel caliber and further highlights the argument that changes in blood vessel caliber are not a driving factor in peripheral sensitization of the trigeminal nerve.
It has previously been suggested that allodynia in migraine may be related to dysfunction in regulatory influences on trigeminocervical neurons, such as the dopaminergic A11 nucleus that might normally inhibit neuronal firing before allodynia has a chance to evolve (Charbit et al., 2007). Repeated activation of the trigeminal nerve, due to the release of inflammatory and other peptides, is thought to be responsible for the peripheral and central sensitization that results in the symptoms of allodynia (Burstein et al., 2000), and one study has shown that intravenous CGRP facilitated responses to vibrissal stimulation by a maximum of 170% (Cumberbatch et al., 1999). Perhaps on the periphery, dopamine receptors have a role in allodynia, with peripheral D1-like receptors playing a role in maintaining the trigeminal nerve activation that leads to its sensitization (Strassman et al., 1996).

It is important to remember though that dopamine also has a high affinity for α-adrenergic receptors that also reside in the vascular smooth muscle (Willems et al., 1985; Missale et al., 1998). Norepinephrine is a major sympathetic neurotransmitter, and it acts on both α- and β-adrenergic receptors, which can be found in blood vessel walls (Keller et al., 1989). A study of the external carotid circulation, illustrated that intravenous dopamine caused vasoconstriction, but when applied with α-adrenoreceptor antagonist phentolamine, vasodilation occurred (Villalon et al., 2003). In the same study, fenoldopam, being D1-like receptor specific, simply caused vasoldilation. In another study, intravenous dopamine inhibited neurogenic dural vasodilation and caused vasoconstriction, but this was reversed to some extent by α2-adrenoreceptor antagonist yohimbine, again suggesting that dopamine also acts at α-adrenergic receptors (Akerman and Goadsby, 2005). Similarly we might find it useful to use adrenergic antagonists to further demonstrate that dopamine is indeed binding to its D1 receptors and not to
adrenergic receptors. Having said this, it remains likely that D₁-like receptors in the vascular bed are being activated by circulating dopamine. Dopamine is a normal constituent of human plasma, accounting for more than 25% that of norepinephrine and approximately equivalent to that of epinephrine (Van Loon and Sole, 1980). The normal quantity of circulating free dopamine is 56 ± 40 pg/mL, norepinephrine is 256 ± 113 pg/mL and epinephrine is 57 ± 41 pg/mL (Marasini et al., 1987). Plasma dopamine originates in sympathetic nerves and adrenal tissue, with the majority originating in sympathetic nerves (Lackovic and Relja, 1983). Events associated with increases in sympathetic activity such as stress, exercise, standing, or hypovolemia are associated with increases in plasma dopamine concentration, although the responses may be of considerably smaller magnitude than those for plasma norepinephrine and epinephrine (Van Loon and Sole, 1980). Additionally D₁ receptors have a widespread distribution in the body, with immunohistochemistry demonstrating the localization of dopamine D₁ and D₅ receptors in the smooth muscle of systemic arteries (Amenta et al., 2002). Vascular D₁-like receptors are located on the smooth muscle of most arterial beds, particularly in the renal and splanchnic arteries, with lesser density in the coronary and cerebral arteries (Goldberg, 1984). This pharmacological and biochemical data supports the existence of a widely distributed dopaminergic vasoactive system (Marasini et al., 1987), and suggests that dopamine must have its own peripheral function, other than binding norepinephrine receptors and being a norepinephrine precursor.

In conclusion our data support the argument that central, but not peripheral, D₂-like receptors play a role in trigeminovascular nociceptive processing. These studies also demonstrate that peripheral D₁-like receptors may contribute to peripheral sensitization. Finally, the data suggest blood vessel caliber has no influence on evoked firing in the TCC or on sensitization of the
trigeminal nerve. Taken together the data provides some insight into the conflicting literature on
the role of dopamine and its receptors in migraine, and offer some possible avenues for
therapeutic advances.
References


Footnotes

The study was funded by a University of California, San Francisco, Neurology start-up grant.
Legends for figures

**Figure 1.** Localization and neuronal characteristics of recording sites in the TCC. 

*A,* The cutaneous receptive field of all neurons studied, was in the first (ophthalmic) division of the trigeminal nerve. 

*B,* An original tracing from a typical unit responding to middle meningeal artery/dural stimulation (arrow represents the stimulus artifact). 

*C,* Locations of recording sites in the TCC for all experiments. Locations were reconstructed from lesions (closed circles) or from microdrive readings (open circles).

**Figure 2.** Example of the blood pressure changes seen immediately post injection of each of the different dopamine receptor specific drugs. Arrows indicate when drug was administered.

**Figure 3.** Effect of D₂-like receptor specific drugs on evoked firing in the TCC, compared to baseline evoked firing, at five minute intervals over forty minutes. 

*A,* Centrally active D₂-like receptor agonist quinpirole. 

*B,* Centrally active D₂-like receptor antagonist S-(−)-eticlopride. 

*C,* Centrally active D₂-like receptor antagonist remoxipride. 

*D,* Peripheral-specific D₂-like receptor antagonist domperidone. 

*E,* quinpirole + domperidone.  

Error bars represent the S.E.M.  *P* < 0.05 significance compared with baseline

**Figure 4.** Effect of D₁-like receptor specific drugs on evoked firing in the TCC, compared to baseline evoked firing, at five minute intervals over forty minutes. 

*A,* Peripheral-specific D₁-like receptor agonist fenoldopam. 

*B,* Centrally active D₁-like receptor agonist A68930. 

*C,* Centrally

Error bars represent the S.E.M. *P < 0.05 significance compared with baseline

**Figure 5.** Post stimulus histograms, illustrating effects of intravenous administration of the D₂-like receptor specific drugs. A, MMA-evoked firing, following intravenous centrally active D₂-like receptor antagonist S-(-)-eticlopride. B, MMA-evoked firing, following intravenous centrally active D₂-like receptor agonist quinpirole. C, MMA-evoked firing, following intravenous centrally active D₂-like receptor agonist quinpirole + peripheral-specific D₂-like receptor antagonist domperidone

**Figure 6.** Peri stimulus histograms, illustrating effects of intravenous administration of the D₁-like receptor specific drugs. A, Noxious pinch and innocuous brush-evoked firing, following intravenous peripheral-specific D₁-like receptor agonist fenoldopam. B, Noxious pinch and innocuous brush-evoked firing, following intravenous centrally active D₁-like receptor agonist A68930.

**Figure 7:** Original tracings showing the effects of A, centrally active D₂-like receptor agonist quinpirole, B, peripheral-specific D₁-like receptor agonist fenoldopam and C, centrally active D₁-like receptor agonist A68930 hydrochloride on arterial blood pressure and dural blood vessel diameter.
Table 1: Summary of all drugs administered intravenously

<table>
<thead>
<tr>
<th>DRUG</th>
<th>DOSE FOR</th>
<th>SPECIFICITY OF DRUG</th>
<th>REFERENCE</th>
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<tr>
<td>Quinpirole hydrochloride</td>
<td>3 mg/kg</td>
<td>Selective D&lt;sub&gt;2&lt;/sub&gt;-like receptor agonist</td>
<td>(Missale et al., 1998)</td>
</tr>
<tr>
<td>S(-)-etilopride hydrochloride</td>
<td>3 mg/kg</td>
<td>Selective D&lt;sub&gt;2&lt;/sub&gt; / D&lt;sub&gt;3&lt;/sub&gt; receptor antagonist</td>
<td>(Missale et al., 1998)</td>
</tr>
<tr>
<td>Remoxipride hydrochloride</td>
<td>8 mg/kg</td>
<td>Standard D&lt;sub&gt;2&lt;/sub&gt; receptor antagonist showing good selectivity over D&lt;sub&gt;3&lt;/sub&gt; and D&lt;sub&gt;4&lt;/sub&gt; receptors</td>
<td>(Ogren and Fuxe, 1988)</td>
</tr>
<tr>
<td>Domperidone hydrochloride</td>
<td>1.5 mg/kg</td>
<td>Selective D&lt;sub&gt;2&lt;/sub&gt;-like receptor antagonist that does not cross the BBB</td>
<td>(Lanfranchi et al., 1985)</td>
</tr>
<tr>
<td>Fenoldapam hydrochloride</td>
<td>1 mg/kg</td>
<td>Selective D&lt;sub&gt;1&lt;/sub&gt;-like receptor agonist that does not cross the BBB</td>
<td>(Flaim et al., 1985; Christie and Smith, 1994)</td>
</tr>
<tr>
<td>A 68930 hydrochloride</td>
<td>1 mg/kg</td>
<td>Selective, full efficacy D&lt;sub&gt;1&lt;/sub&gt;-like dopamine receptor agonist</td>
<td>(Christie and Smith, 1994)</td>
</tr>
<tr>
<td>Drug Name</td>
<td>Dose</td>
<td>Action</td>
<td>Reference</td>
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<td>-------------------------------------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Dihydrexidine</td>
<td>3 mg/kg</td>
<td>Selective, full efficacy D&lt;sub&gt;1&lt;/sub&gt;-like dopamine receptor agonist. No agonist activity at peripheral D&lt;sub&gt;2&lt;/sub&gt; receptors or adrenoceptors.</td>
<td>Brewster et al., 1990</td>
</tr>
<tr>
<td>SCH 23390 hydrochloride</td>
<td>1 mg/kg</td>
<td>Potent D&lt;sub&gt;1&lt;/sub&gt;-like receptor antagonist. Also D&lt;sub&gt;2&lt;/sub&gt;, D&lt;sub&gt;3&lt;/sub&gt; &amp; D&lt;sub&gt;4&lt;/sub&gt; receptors 10000 x less. Also agonist at 5-HT&lt;sub&gt;1C/2C&lt;/sub&gt; receptors in vitro.</td>
<td>Missale et al., 1998</td>
</tr>
</tbody>
</table>
Figure 4

A. fenoldopam

B. A68930

C. dihydroxidine

D. SCH23390

E. water

Percentage of baseline firing

Time (min)

Baseline | Post iv peripheral D1R agonist

Baseline | Post iv central D1R agonist

Baseline | Post iv saline

Baseline | Post iv central D1R antagonist

Baseline | Post iv saline

MMA | Noxious pinch | Innocuous brush

MMA | Noxious pinch | Innocuous brush

MMA | Noxious pinch | Innocuous brush

MMA | Noxious pinch | Innocuous brush
Figure 7

A. Quinpirole

B. Fenoldopam

C. A68930

D. Dihydrexidine