Comparison of the Effects of Central and Peripheral Dopamine Receptor Activation on Evoked Firing in the Trigeminocervical Complex

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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 ophthalmic dermatome, D_{1}- or D_{2}-like receptor agonists or antagonists were administered intravenously and the effect on firing was determined. In addition, with use of intravitral microscopy, we monitored changes in dural vessel diameter in response to varying doses of D_{1}- or D_{2}-like receptor agonists to determine whether their effects were related to blood vessel caliber. The central D_{2}-like receptor agonist quinpirole hydrochloride inhibited firing in the TCC evoked by stimulation of the MMA. Conversely, the central D_{2}-like receptor antagonists, eticlopride hydrochloride and remoxipride hydrochloride, facilitated MMA-evoked firing and also firing evoked by noxious and innocuous stimulation of the ophthalmic dermatome. Both the peripheral D_{1}-like receptor agonist fenoldopam and the central D_{1}-like receptor antagonists cis-(\pm)-1-(aminomethyl)-3,4-dihydro-2-benzopyran-5,6-diol hydrochloride (A68930 hydrochloride) and dihydrexidine facilitated innocuous brush-evoked firing, with A68930 hydrochloride having the greatest effect. The data suggest that dopamine binding to peripheral D_{1}-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. In addition, these studies maintain that central D_{2}-like receptors inhibit trigeminocervical neurons, and may provide insight into the conflicting literature on the role of dopamine and its receptors in migraine.

It is generally accepted that migraine involves activation, or the perception of activation, of trigeminovascular afferents (Goadsby et al., 2002). Dopamine has certainly been implicated among the other substances putatively involved in migraine (Peroutka, 1997; Akerman and Goadsby, 2007). Recently, dopamine was found to attenuate noxious or nonnoxious stimulation in the trigeminocervical complex (TCC) activated by durovascular nociceptive stimulation (Bergot et al., 2007). Furthermore, immunocytochemistry demonstrated that D_{1} and D_{2} dopamine receptors can be identified in the TCC (Bergot et al., 2007). In addition, the A11 dopaminergic nucleus, which provides the only known source of descending dopaminergic innervation for the spinal gray matter (Skagerberg et al., 1982), modulates trigeminal processing and this response is reversed by a D_{2}-like receptor antagonist (Charbit et al., 2007). It has therefore been hypothesized that dopamine binds to inhibitory D_{2}-like receptors that exist postsynaptically on second-order neurons in the TCC, and thus inhibits the rostral transmission of nociceptive signals.

In contrast to these experimental data, there is evidence suggesting that dopamine blockade is useful in migraine. Most compelling is the fact that D_{2}-like receptor antagonists, such as prochlorperazine (Jones et al., 1996), metoclopramide (Colman et al., 2004), droperidol (Silberstein et al., 2003), and 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] (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 vasodilation 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 antinoceptive at central D₁ and D₂-like receptors, or at other sites in the central nervous system.

The aim of this study was to compare, with use of electrophysiological techniques, the effects of intravenously administered central and peripheral dopamine receptor agonists and antagonists on evoked firing in the TCC. In addition, using intravital microscopy, we looked at the response on dural blood vessel caliber of varying doses of (−)-quinpirole hydrochloride ([4αR-trans]-4,4α,5,6,7,8α,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline hydrochloride), fenoldopam [6-chloro-2,3,4,5-tetrahydro-1(4-hydroxyphenyl)-1H-3-benzazepine-7,8-diol], and A68930 hydrochloride to determine whether the observed changes of these D₂ and D₁-like receptor agonists on dural vessel caliber contribute to their effects on trigeminovascular nociceptive processing.

Materials and Methods

Electrophysiology Experiments

Surgery. Fifty male Sprague-Dawley rats (300–500 g) were anesthetized throughout the experiments. Anesthesia was induced with intraperitoneal pentobarbital sodium (Sigma Chemical, Poole, Dorset, UK), 60 mg·kg⁻¹ in saline, and maintained by slow intravenous infusion of propofol at 40 μg·kg⁻¹·h⁻¹ (Rapinovet; Schering-Plough Animal Health, Boxmeer, The Netherlands). A sufficient depth of anesthesia was judged by the absence of withdrawal reflexes in the nonparalyzed state or by fluctuations in blood pressure during muscular paralysis. The left femoral artery and vein were exposed and cannulated for recording blood pressure and intravenous infusion of anesthetic, respectively. The rats were paralyzed with 1 mg/kg pancuronium bromide (Pavulon; Organon, Cambridge, UK) and ventilated with oxygen-enriched air, 2 ml, 80 to 100 strokes per min (model 7025; Ugo Basile, Comerio, Italy). End-tidal CO₂ was monitored (Capstar 100; CWE Inc., Ardmore, PA) and maintained between 2.8 and 4.9%. Body temperature was monitored via a rectal thermometer and kept within physiological range at 36.9 to 37.9°C, between 2.8 and 4.9%. Body temperature was monitored via a rectal thermometer and kept within physiological range at 36.9 to 37.9°C, using a homeothermic blanket system (homeothermic blanket system for rodents; Harvard Apparatus, Holliston, MA). At the end of the experiment, animals were sacrificed with an overdose of anesthetic (pentobarbital, 0.7 ml of 200 mg/ml).

The rats were placed in a stereotaxic frame (model 1600 stereotaxic frame; David Kopf Instruments, Tujunga, CA), the skull was exposed, and a craniotomy of the left parietal bone was performed with a saline-cooled drill (MF-Perfecta with 945 handpiece; W&H, St. Albans, Hertfordshire, UK), 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 performed. 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) 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 to 15 V was applied (Grass S88; Grass Instruments, Quincy, MA). Extracellular recordings were made in the ipsilateral 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 to 2 MΩ and tip diameter of 0.5 μm (tungsten metal microelectrode; Harvard Apparatus). The recording electrode, suspended on a piezoelectric microdrive (EXPO-Burleigh Products Inc., Victor, NY) attached to the stereotaxic frame, was driven by a controller (EXPO Controller 8200 Inchworm; EXPO-Burleigh Products Inc.). 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, 2000×; Digitimer, Welwyn Garden City, UK), through filters (Neurolog NL125; bandwidth 300 Hz to 20 kHz) and a 50-Hz noise eliminator (Hum Bug; Quest Scientific, North Vancouver, Canada), and through a second-stage amplifier (Neurolog NL106; gain, 200–999×). The filtered and amplified electrical signal was routed to a loudspeaker, via a power amplifier (Neurolog NL120), and was displayed on analog and digital-storage oscilloscopes (Goldstar, LG Precision, Seoul City, South Korea and Metrix Electronics, Hampshire, UK, respectively), which displayed a single voltage pulse (or spike) of given height and duration in response to each action potential whose height fell within a preset window of a window discriminator. The window discriminator output data were then displayed on a computer as either a peristimulus histogram or a poststimulus histogram. The signal was also fed to an analog-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. Peristimulus histograms were used for observing the evoked firing in response to noxious and innocuous cutaneous stimulations (pinch and brush), and from the dura mater, using noxious pinch and innocuous brush, and from the MMA. The filtered and amplified electrical signal was routed to a loudspeaker, via a power amplifier (Neurolog NL120), and was displayed on analog and digital-storage oscilloscopes (Goldstar, LG Precision, Seoul City, South Korea and Metrix Electronics, Hampshire, UK, respectively), which displayed a single voltage pulse (or spike) of given height and duration in response to each action potential whose height fell within a preset window of a window discriminator. The window discriminator output data were then displayed on a computer as either a peristimulus histogram or a poststimulus histogram. The signal was also fed to an analog-to-digital converter (Cambridge Electronic Design, Cambridge, UK) and then to a personal computer.

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. After baseline recordings, drugs were administered via the cannulated femoral vein, at volumes of 0.4 to 0.8 ml, and the effect of each drug on baseline evoked firing was monitored at 5-min intervals for 40 min.

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 were expressed as a percentage of the mean of those baselines. Data were assessed by use of an analysis of variance for repeated measures test. If this was statistically significant, the data were also grouped into individual time points and analyzed by use of a Student’s paired t test with Bonferroni post hoc correction for multiple comparisons. Summary data are expressed as the mean ± S.E.M. Significance was assessed at the P < 0.05 level.
TABLE 1
Summary of all drugs administered intravenously

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose for Electrophysiology Experiments</th>
<th>Specificity of Drug</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinpirole hydrochloride (Tocris Bioscience, Bristol, UK)</td>
<td>3 mg/kg</td>
<td>Selective D_2-like receptor agonist</td>
<td>Missale et al., 1998</td>
</tr>
<tr>
<td>S-(−)-Eticlopride hydrochloride (Tocris Bioscience)</td>
<td>3 mg/kg</td>
<td>Selective D_2/D_3 receptor antagonist. D_4 receptor antagonist, less.</td>
<td>Missale et al., 1998</td>
</tr>
<tr>
<td>Remoxipride hydrochloride</td>
<td>8 mg/kg</td>
<td>Standard D_2 receptor antagonist showing good selectivity over D_3 and D_4 receptors.</td>
<td>Ogren and Fuxe, 1988</td>
</tr>
<tr>
<td>Domperidone (Tocris Bioscience)</td>
<td>1.5 mg/kg</td>
<td>Selective D_2-like receptor antagonist that does not cross the BBB. Also α-adrenoceptor antagonist in vitro.</td>
<td>Lanfranchi et al., 1985</td>
</tr>
<tr>
<td>Fenoldopam hydrochloride (Tocris Bioscience)</td>
<td>1 mg/kg</td>
<td>Selective D_1-like receptor agonist that does not cross the BBB. Also α-adrenoceptor antagonist in vitro.</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_2-like dopamine receptor agonist</td>
<td>Christie and Smith, 1994</td>
</tr>
<tr>
<td>Dihydrexidine hydrochloride (Tocris Bioscience)</td>
<td>3 mg/kg</td>
<td>Selective, full-efficacy D_1-like dopamine receptor agonist. No agonist activity at peripheral D_3 receptors or adrenceptors.</td>
<td>Brewster et al., 1990</td>
</tr>
<tr>
<td>SCH 23390 hydrochloride (Tocris Bioscience)</td>
<td>1 mg/kg</td>
<td>Potent D_1-like receptor antagonist. Also D_2, D_3, and D_4 receptors 10,000 times less. Also agonist at 5-HT_1C/C receptor in vitro.</td>
<td>Missale et al., 1998</td>
</tr>
</tbody>
</table>

BBB, blood-brain barrier.

Postoperative Examination of Tissue. At the end of the experiment and just before 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 s). After terminal anesthesia, the brain was removed and 60-μm-thick coronal sections were cut and visualized under the light microscope (Axioplan Microscope; Carl Zeiss GmbH, Jena, Germany), 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 trigemino-cervical complex were related to dural blood vessel changes, in particular, for D_2- and D_1-like receptor agonists. We performed a separate set of experiments, in which rats were given varying intravenous doses of the centrally acting D_2-like receptor agonist quinpirole hydrochloride, the peripheral D_1-like receptor agonist fenoldopam hydrochloride, and the centrally acting selective and full D_1-like receptor agonists A68930 hydrochloride and dihydrexidine [(±)-trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine], and the effects on dural blood-vessel diameter and 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 at the University of California, San Francisco. All animals (n = 14) were anesthetized with pentobarbital (Nembutal 80 mg/ml, initially 60 mg/kg) and maintained on an infusion at 40 mg/kg/h. 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 was similar to the electrophysiological setup, except drilling was used to create a thinned cranial window, approximately 5 × 5 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 (magnification, 80–450 ×), connected to an intravital microscope (MS-500C MicroScopeman; Moritex, Cambridge, UK) was positioned above the cranial window and the image of the dural artery was displayed on a standard television monitor. Dural blood vessel diameter was continuously measured using a video dimension analyzer (Living Systems Instrumentation, Burlington, VT). For these intravital microscopy experiments, no further surgery was required. The animal was left for up to 1 h 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 5 min, followed by intravenous administration of the drug. When blood vessel diameter and blood pressure returned to normal, and no less than 5 min after injection, the next drug dose was given. The peripheral D_1-like receptor agonist, fenoldopam hydrochloride, was administered in doses of 3, 10, 30 μg/kg, and 1 mg/kg i.v., and the effect of the drugs on dural blood vessel caliber was observed. The centrally acting D_2-like receptor agonist, quinpirole hydrochloride, was administered in doses of 50, 150, and 300 μg/kg i.v. and 3 mg/kg i.v. The centrally acting D_1-like receptor agonist, A68930 hydrochloride, was administered in doses of 100 and 300 μg/kg i.v. and 1 mg/kg i.v., and dihydrexidine hydrochloride was administered in doses of 0.5, 1, and 3 mg/kg i.v.

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 standardize the dural vessel measurement; therefore, the change in dural vessel diameter was reported as a percentage change from prestimulation diameter (baseline maximal stimulation response = 100%). The vessels typically ranged from 120 to 150 μm in diameter. Summary data, mean ± S.E.M., are therefore expressed as a percentage of the mean of the baseline diameter. Data were analyzed by use of a Student’s paired t test. Significance was assessed at the P < 0.05 level.
Results

Electrophysiological Data

Extracellular recordings in the TCC were made from a total of 50 wide-dynamic-range neurons, responsive to dural/MMA stimulation (8–15 V, 0.5 ms, 0.6 Hz), and with cutaneous receptive fields in the ophthalamic division of the trigeminal nerve (Fig. 1A). Neurons responding to MMA stimulation responded at latencies of 6 to 20 ms, and hence were receiving input from Aδ fibers (Fig. 1B). The locations of these neurons ranged from superficial to deep layers (laminae I–V) of the dorsal horn of the TCC, at depths ranging from 135 to 1106 μm (Fig. 1C).

Effect of Intravenous D₂-Like Receptor Specific Drugs on Evoked Firing. Central D₂-like receptor agonist.

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 to 30 mm Hg, which fully recovered after 60 s (Fig. 2). MMA-evoked firing was significantly decreased by 17 ± 3% (F₁,₅ = 2.47; P < 0.05; n = 7) below baseline over 40 min. The greatest point of inhibition was at 5 min, by 25 ± 9% (t₆ = 2.94; P < 0.05; n = 7) below baseline firing. Noxious pinch- and innocuous brush-evoked firing remained at approximately baseline at all time points from 5 to 40 min, with no significance across the cohort (Figs. 3A and 4A).

Central D₂-like receptor antagonist. After administration of the central D₂-like receptor antagonists, S(-)-eticlopride hydrochloride [3-chloro-5-ethyl-N-[(2S)-1-ethyl-2-pyrrolidinylmethyl]-6-hydroxy-2-methoxy-benzamide hydrochloride] (3 mg/kg; n = 5) or remoxipride hydrochloride [[S]-(-)-3-bromo-N-[(1-ethyl-2-pyrrolidinyl)methyl]2,6-dimethoxybenzamide hydrochloride] (8 mg/kg; n = 7), there was a transient drop in blood pressure by 15 to 35 mm Hg that fully recovered after 12 to 14 s for eticlopride hydrochloride and after 45 to 50 s for remoxipride hydrochloride (Fig. 2). With eticlopride hydrochloride, MMA-evoked firing significantly increased by 14 ± 3% (F₅,₃₀ = 2.73; P < 0.05; n = 7) above baseline over 25 min, with maximal facilitation at 5 min by 17 ± 5% (t₆ = −3.74; P < 0.05; n = 7) above baseline firing. Noxious pinch-evoked firing showed a trend for facilitation, although this was not significant over any time period, and innocuous brush-evoked firing remained at approximately baseline throughout (Figs. 3B and 4B). With remoxipride hydrochloride, MMA-evoked firing significantly increased by 20 ± 4% (F₃,₁₅ = 6.84; P < 0.05; n = 7) above baseline over 15 min, with maximal facilitation at 10 min by 32 ± 4% (t₆ = −7.43; P < 0.05; n = 7). Noxious pinch-evoked firing significantly increased by 28 ± 6% (F₄,₂₄ = 2.89; P < 0.05; n = 7), above baseline over 20 min with maximal facilitation at 5 min by 46 ± 18% (t₆ = −2.54; P < 0.05; n = 7). Innocuous brush-evoked firing significantly increased by 24 ± 3% (F₅,₃₀ = 2.92; P < 0.05; n = 7) above baseline over 25 min, with maximal facilitation at 25 min by 29 ± 6% (t₆ = −4.92; P < 0.05; n = 7) (Figs. 3C and 4C).

Peripheral D₂-like receptor antagonist. The peripheral D₂-like receptor antagonist, domperidone (1.5 mg/kg; n = 5), had no effect on blood pressure (Fig. 2), and MMA- and noxious pinch- and innocuous brush-evoked firing remained at approximately baseline at all time points from 5 to 40 min, with no significance across the cohort (Fig. 3D).

Central D₂-like receptor agonist and peripheral D₂-like receptor antagonist. After administration of the central D₂-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 to 30 mm Hg that lasted no more than 60 s (Fig. 2). MMA-evoked firing significantly decreased by 13 ± 2% (F₃,₁₈,₃ = 2.63; P < 0.05; n = 7) below baseline over a 40-min cohort. The greatest point of inhibition was at 5 min, by 21 ± 5% (t₆ = 3.89; P < 0.05; n = 7) below baseline firing. Noxious pinch- and innocuous brush-evoked firing remained at approximately baseline at all time points, with no significance across the cohort (Figs. 3E and 4D).

Effect of Intravenous D₁-Like Receptor Specific Drugs on Evoked Firing. After 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 to 40 mm Hg that fully recovered after 65 to 125 s (Fig. 2). Innocuous brush-evoked firing significantly increased by 20 ± 3% (F₂,₉,₂₀,₅ = 2.63; P < 0.05; n = 8) above baseline firing over a 40-min cohort. MMA- and noxious pinch-evoked firing remained around baseline at all time points from 5 to 40 min, with no significance across the cohort (Figs. 5A and 6A).

On administration of the centrally active, full, and irreversible D₁-like receptor agonist, A68930 hydrochloride (1

Fig. 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 (●) or from microdrive readings (○).
mg/kg; n = 8), there was an immediate rise in blood pressure by 30 to 60 mm Hg, that lasted for 75 to 175 s, although it only fully recovered after approximately 10 min (Fig. 2). In contrast, 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.

Fig. 2. Example of the blood pressure changes seen immediately after injection of each of the different dopamine receptor-specific drugs. Arrows indicate when drug was administered. BP, blood pressure.
pressure by 28 to 36 mm Hg that lasted for 12 to 18 min (Fig. 2). Both A68930 hydrochloride and dihydrexidine facilitated innocuous brush-evoked firing. A68930 hydrochloride caused innocuous brush-evoked firing to significantly increase by 36 ± 4% ($F_{2.49} = 2.29; P < 0.05; n = 8$) above baseline across a 35-min cohort, starting at 10 min after injection. MMA-evoked firing remained at approximately baseline at all time points from 5 to 40 min, whereas noxious pinch-evoked firing
showed a trend for facilitation, although this was not significant over any time period (Figs. 5B and 6B). Dihydrexidine hydrochloride caused innocuous brush-evoked firing to significantly increase by $20 \pm 4\%$ ($F_{7,49} = 3.39; P < 0.05; n = 8$) above baseline, and noxious pinch-evoked firing to significantly increase by $23 \pm 3\%$ ($F_{7,49} = 2.34; P < 0.05; n = 8$)

Fig. 4. Poststimulus histograms, illustrating effects of intravenous administration of the D2-like receptor-specific drugs. A, MMA-evoked firing, after intravenous administration of centrally active D2-like receptor agonist quinpirole. B, MMA-evoked firing, after intravenous administration of centrally active D2-like receptor antagonist S-(-)-eticlopride. C, MMA-evoked firing, after intravenous administration of centrally active D2-like receptor antagonist remoxipride. D, MMA-evoked firing, after intravenous administration of centrally active D2-like receptor agonist quinpirole + peripheral-specific D2-like receptor antagonist domperidone.
above baseline, both across a 35-min cohort, starting at 5 min after injection (Figs. 5C and 6C).

The central D₁-like receptor antagonist, SCH23390 (1 mg/kg; n = 5) had no effect on blood pressure (Fig. 2), and MMA-, noxious pinch-, and innocuous brush-evoked firing remained at approximately baseline at all time points from 5 to 40 min, with no significance across the cohort (Fig. 5D). The intravenous water control did not significantly affect MMA-, noxious
pinch-, or innocuous brush-evoked firing in the TCC from 5 to 40 min (Fig. 5E).

**Intravital Microscopy.** The centrally acting D2-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% (t4 = -3.69; *, P < 0.05; n = 5), 55 ± 6% (t4 = -9.41; *, P < 0.05; n = 5), 68 ± 9% (t4 = -8.08; *, P < 0.05; n = 5), and 83 ± 10% (t4 = -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 (Fig. 7A).

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% (t4 = -5.75; *, P < 0.05; n = 5) and 54 ± 12% (t4 = -4.74; *, P < 0.05; n = 5).
in blood vessel diameter at all doses, 100 μg/kg, produced a significant dose-dependent decrease 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 (Fig. 7C).

The centrally acting D_1-like receptor agonist dihydrexidine hydrochloride also produced a significant dose-dependent decrease in blood vessel diameter at all doses, 0.5, 1, 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 (Fig. 7D).

Discussion

The data demonstrate that dopamine D_2 receptor agonists that cross the blood-brain barrier inhibit neurons in the TCC with dural inputs, and D_2 receptor antagonists facilitate these neurons. In contrast, peripherally restricted D_2 receptor antagonists have no effect on TCC responses alone and do not alter the effect of centrally active D_2 receptor agonists. D_1 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 D_1 receptors in sensitization.

D_2-Like Receptor Effects. The D_2-like receptor agonist, quinpirole, and quinpirole + peripheral D_2-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 antinoceptive properties at D_2-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 trigeminocephalic neuronal activity.

Both eticlopride and remoxipride, D_2-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. Eti clopride and remoxipride, both substituted benzamides, are atypical antipsychotics (Högberg et al., 1987). Ideally, we would have liked to use a typical D_2-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 resulting 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 D_2-like receptor (Högberg et al., 1987). Remoxipride, for instance, has been found to be considerably (50-fold) more potent than sulpiride in antagonizing 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 noxious stimuli, which is abolished when the D\textsubscript{2}-like receptors are blocked.

Dopaminedone alone had no effect on evoked firing in the TCC, suggesting that peripheral D\textsubscript{2}-like receptors do not play a role in trigeminal nerve activation. Dopaminedone, however, has been shown to prevent the occurrence of migraine when taken during the premonitory phase of the attack (Waelkens, 1984). Peripheral D\textsubscript{2}-like receptors are located presynaptically on sympathetic nerves, where they inhibit the release of norepinephrine (Kohli et al., 1983), suggesting that D\textsubscript{2} receptors are indirect vasodilators by inhibition of vasoconstriction (Kohli et al., 1983; Lahhou, 1998; Amenta et al., 2002). In addition, D\textsubscript{2}-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 because blockade of D\textsubscript{2}-like receptors has been shown to relieve these premonitory symptoms (Waelkens, 1984). In contrast, D\textsubscript{3}-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 trigeminocephalic complex?

D\textsubscript{1}-Like Receptor Effects. Both the centrally active (A68930 and dihydrexidine) and the peripheral only (fenoldopam) D\textsubscript{1}-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 D\textsubscript{1}-like dopamine receptors and binds with moderate antagonist affinity to \(\alpha\)-adrenoceptors (Ohlstein et al., 1985). It has no significant affinity for D\textsubscript{2}-like receptors, \(\alpha\)- and \(\beta\)-adrenoceptors, 5HT\textsubscript{1} and 5HT\textsubscript{2} 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 D\textsubscript{1}-like receptor agonist (Flaim et al., 1985). A68930 is a selective, full-eficacy, irreversible D\textsubscript{1}-like dopamine receptor agonist (DeNinno et al., 1990; Grenader and Healy, 1992) that binds very weakly to D\textsubscript{2}-like receptors, and is virtually inactive at \(\alpha\)- and \(\beta\)-adrenoceptors. It readily crosses the blood-brain barrier, and so is considered a central D\textsubscript{1}-like receptor agonist (Kebabian et al., 1990). Dihydrexidine is a selective, fully efficacious, and reversible D\textsubscript{1}-like dopamine receptor agonist, with no agonist activity at peripheral D\textsubscript{2} receptors or adrenoceptors. Dihydrexidine seems to be fully bioavailable in brain and exhibits profound anti-parkinsonian effects in vivo (Brewster et al., 1990). The fact that both the centrally active and the peripheral only D\textsubscript{1}-like receptor agonists facilitated innocuous brush-evoked firing suggests that the facilitatory action is at least at peripheral D\textsubscript{1}-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 12 times more potent than fenoldopam (Christie and Smith, 1994), and approximately 13 times more potent than dihydrexidine (Watts et al., 1995). However, the high doses used in these experiments most likely saturated the D\textsubscript{1} receptor. Therefore, the observed differences in the degree of facilitation after intravenous administration of these D\textsubscript{1} receptor agonists was probably due, not to potency of the drugs, but to some other feature such as off-target action, functional selectivity at D\textsubscript{1} signaling systems, or differential functional actions at D\textsubscript{1} versus D\textsubscript{2} receptors.

D\textsubscript{1}-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 by use of intravital microscopy, and it is interesting to note that, although 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 trigeminofacial nociceptive 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 calcitonin gene-related peptide 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 D\textsubscript{1}-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, however, that dopamine also has a high affinity for \(\alpha\)-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 \(\alpha\)- and \(\beta\)-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 \(\alpha\)-adrenoceptor antagonist phentolamine, vasodilation occurred (Villalón et al., 2003). In the same study, fenoldopam, being D\textsubscript{1}-like receptor-specific, simply caused vasodilation. In another study, intravenous dopamine inhibited neurogenic dural vasodilation and caused vasoconstriction, but this was reversed to some extent by the \(\alpha\)-adrenoceptor antagonist yohimbine, again suggesting that dopamine also acts at \(\alpha\)-adrenergic receptors (Akerman and Goadsby, 2005). Likewise, we might find it useful to use adrenergic antagonists to further demonstrate that dopamine is indeed binding to its D\textsubscript{1} receptors and not to adrenergic receptors. Having said this, it remains likely that D\textsubscript{1}-like receptors in the vascular bed are being activated by circulating dopamine. Dopamine is a normal constituent of human plasma, with levels at 25% of norepinephrine levels and approximately equivalent to epinephrine levels (Van Loon and Sole, 1980). The normal quantity of circulating free dopamine is 56 \pm 40 pg/ml, norepinephrine is 256 \pm 113 pg/ml, and epinephrine is 57 \pm 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). In addition, D1 receptors have a widespread distribution in the body, with immunohistochemistry demonstrating the localization of dopamine D1 and D3 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, in particular, in the renal and splanchic arteries, with lesser density in the coronary and cerebral arteries (Goldberg, 1984). These pharmacological and biochemical data support the existence of a widely distributed dopaminergic vasoactive system (Marasini et al., 1987), and suggest 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 that blood vessel caliber has no influence on evoked firing in the TCC or on sensitization of the trigeminal nerve. Taken together, the data provide 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


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