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under normoxic conditions, SD occurs during migraine aura where it

precedes migraine pain but does not damage tissue. During stroke and

head trauma, however, SD can arise repeatedly near the site of injury

and may promote neuronal damage. We developed a superfused brain

slice preparation that can repeatedly support robust SD during im-

aging and electrophysiological recording to test drugs that may block

SD. Submerged rat neocortical slices were briefly exposed to artificial
cerebrospinal fluid (ACSF) with KCl elevated to 26 mM. SD was

evoked within 2 min, recorded in layers II/III both as a negative DC

shift and as a propagating front of elevated light transmittance (LT)

representing transient cell swelling in all cortical layers. An SD

episode was initiated focally and could be repeatedly evoked and

imaged with no damage to slices. As reported in vivo, pretreatment

with one of several N-methyl-D-aspartate (NMDA) receptor antag-

onists blocked SD, but a non-NMDA glutamate receptor antagonist

(CNQX) had no effect. NMDA receptor (NMDAR) activation does

not initiate SD nor are NMDAR antagonists tolerated therapeutically

so we searched for more efficacious drugs to block SD generation.

Pretreatment with the sigma-one receptor (σ1R) agonists dextro-

methorphan (10–100 μM), carbetapentane (100 μM), or 4-IBP (30

μM) blocked SD, even when KCl exposure was extended beyond 5

min. The block was independent of NMDA receptor antagonism. Two

σ1R antagonists ([+−]-3PPP and BD-1063) removed this block but had

no effect upon SD alone. Remarkably, the σ1R agonists also substan-
tially reduced general cell swelling evoked by bath application of 26

mM KCl. More potent σ1R ligands that are therapeutically tolerated

could prove useful in reducing SD associated with migraine and be of

potential use in stroke or head trauma.

INTRODUCTION

Migraine is an incapacitating headache that often arises unilaterally and is commonly accompanied by nausea and hypersensitivity to light or sound. Headache pain probably results from neurogenic inflammation at the endings of meningeal pain afferents, increasing impulse traffic along cranial nerve V and into the brain stem (Moskowitz and Cutrer 1994). The spinal nucleus of V relays to the thalamus and from there to the cortex where pain is perceived. The widely accepted neurogenic concept proposes that migraine is initially a CNS dysfunction, the associated vascular phenomena being secondary. For 10–20% of migraineurs, the pain is preceded by distinct perceptual symptoms (Russell and Olesen 1996). The aura is typically reported as a moving blind spot or a creeping band of “pins and needles” lasting 20–30 min, the result of a migrating inactivation of sensory cortex. Migraine aura is probably under-reported by patients because when association cortex is the substrate, there may be no perceived deficit. It is therefore possible that aura activates the pathways responsible for the subsequent migraine pain (Donnet and Bartolomei 1997; Gordon 1989; Lauritzen 1987; Strupp et al. 1998). There is recent evidence for (Bolay et al. 2002; Lauritzen 2001) and against (Ebersberger et al. 2001; Lambert et al. 1999) this cause-and-effect argument. In either case, aura and headache are clearly temporally linked manifestations of migraine, so there is a therapeutic rationale for understanding the onset of the aura. Migraine therapies have commonly focused on treating the pain at the meningeal site, temporally downstream from aura generation.

The migrating cortical inactivation known as spreading depression (SD) is likely responsible for the marching sensory deficit often dominating migraine aura. Recently the shifting scotoma in the visual field was correlated with the functional magnetic resonance imaging (fMRI) signal moving along the visual cortex (Hadjikhani et al. 2001). Additional clinical evidence that SD occurs in humans is obtained from PET scanning (Dienier 1997; Woods et al. 1994), magnetoencephalography (Bowyer et al. 2001; Wijesinghe et al. 1998), evoked visual potentials (Shibata et al. 1998), and diffusion weighted MRI (Strupp et al. 1998). SD then, is considered the physiological event responsible for migraine aura and is a possible target for the treatment and prevention of migraine pain (Cutrer et al. 2000).

SD is a profound depolarization of neurons and glia lasting 1–2 min (Somjen 2001). It slowly migrates at 2–5 mm/min across gray matter. The cerebral cortex, where it was first measured as a propagating wave of electrical silence (Leao 1944, 1951) is particularly susceptible. The SD event, like the aura, does not induce neural injury (Lauritzen 1987; Nedergaard and Hansen 1988). A recurring wave of SD can be induced in rat hippocampal slices (Somjen et al. 1992) and in human neocortical slices (Gorji et al. 2001). Repeated SD events have also been reported in submerged slices from rat neocortex (Footit and Newberry 1998).

SD in normally metabolizing cortical tissue (induced by focal K+ mechanical or tetanic stimulation) is blocked by N-methyl-D-aspartate receptor (NMDAR) antagonists (but not by non-NMDAR antagonists) as reported in vivo (Hernandez-Caceres et al. 1987; Marrannes et al. 1988; Lauritzen and...
Hansen 1992; Nellgard and Wieloch 1992; Koroleva et al. 1998) and in hippocampal and neocortical slices (Somjen 2001; Footitt and Newberry 1998). Note that NMDAR participation does not necessarily imply causation (Obrenovitch 2001). In the current study, we test several glutamate receptor antagonists to confirm NMDAR-mediated antagonism of SD in our neocortical slice preparation. In addition, we have recently reported that certain sigma-one receptor (\(\sigma_1\)R) ligands are effective in preventing the propagating wave of anoxic depolarization (AD) (Anderson et al. 2000). This wave arises under simulated ischemia and, if unchallenged, leaves cells damaged in its wake (Obeidat and Andrew 1998; Jarvis et al. 2001; Joshi and Andrew 2001). Similarities between normoxic and hypoxic SD have been noted (Aitken et al. 1998). We have proposed that AD and SD are related phenomena whose ultimate effect is dependent upon the metabolic state of the gray matter through which the signal propagates (Andrew et al. 2002). For this reason, we also examine here \(\sigma_1\)R ligands as potential SD blockers.

There are at least two types of sigma receptor (\(\sigma_1\)R and \(\sigma_2\)R), both distributed throughout the body and CNS (Quirion et al. 1992; Marrazo et al. 2001; Maurice et al. 2001). Their exact biological role is unknown although the \(\sigma_2\)R has been cloned (Hanner et al. 1996) and studied in Xenopus oocytes (Aydar et al. 2002). The designation of \(\sigma_1\)R ligands as “agonists” or “antagonists” depends on the utilized bioassay, which are varied and numerous, and remains tentative until the actual function of the sigma receptor is better understood.

The \(\sigma_1\)R ligands, identified by receptor binding studies, mediate a variety of proposed biological effects including neuroprotection, often ascribed to some degree of NMDAR cross reactivity (Takahashi et al. 1996; Senda et al. 1998; Nishikawa et al. 2000). However, the role of NMDARs in neuroprotection is being increasingly questioned (Jarvis et al. 2001; Obrenovitch 2001; Obrenovitch and Urenjak 1997; but see Palmer 2001). Certain \(\sigma_1\)R ligands reduce ischemic damage in vivo (George et al. 1988; Prince and Feeser 1988) and in vitro (Wong et al. 1988; Church et al. 1991), and there is also evidence for protection independent of NMDARs (Lockhart et al. 1995; Klette et al. 1997). Here we test \(\sigma_1\)R ligands for their ability to prevent SD independent of NMDAR activity and as possible alternatives to NMDAR antagonists, the latter being poorly tolerated clinically.

**METHODS**

**Neocortical slice preparation**

Male Sprague-Dawley rats, 21–28 days old (Charles River, St. Constant, Quebec) were housed in a controlled environment (22 ± 1°C, 12 h light:12 h dark cycle) with food (Purina rat chow) and water supplied ad libitum. Rats were placed in a rodent restrainer (DecapitCone; Braintree Scientific, Inc.) and guillotined. The brain was excised and placed in ice-cold oxygenated (95% O\(_2\)-5% CO\(_2\)) artificial cerebrospinal fluid (ACSF). Coronal slices (400 \(\mu\)m) were taken from the frontal-parietal region of the neocortex using a vibrating blade microtome (Leica VT1000S). Five to seven slices were transferred to a submerged net in a beaker containing ACSF gassed with O\(_2\)-CO\(_2\) at 22°C. The slices were then slowly warmed over one hour to 32 ± 1°C prior to experimentation.

**Experimental solutions and drugs**

The ACSF contained (in mM) 120 NaCl; 3.3 KCl; 26 NaHCO\(_3\); 1.3 MgSO\(_4\); 7H\(_2\)O; 1.2 NaH\(_2\)PO\(_4\); 1.8 CaCl\(_2\); and 11 g/lucose. All were dissolved in double-distilled water at pH 7.3–7.4. The ACSF was used for incubation and as a vehicle to administer experimental solutions. For high-K\(^+\) ACSF, 26 mM KC1 replaced equimolar NaCl. The following drugs were added to control ACSF or elevated-K\(_+\) ACSF as required: kynurenic acid (2 mM), ib-2-amino-5-phosphonovaleric acid (AP-5, 50 or 100 \(\mu\)M), 6-cyano-7-nitroquinoline-2,3-(1H,4H)-dione (CNQX, 10 \(\mu\)M), MK-801 (100 \(\mu\)M), dextromethorphan (DM, 1–100 \(\mu\)M), carbetapentane (CP, 100 \(\mu\)M), N-(N-benzylpiperidine-4-yl)-4-iodobenzamide (4-IBP, 30 \(\mu\)M), 3-(3-hydroxyphenyl)-N-(1-propyl)piperidine-[R-3-P, 100 \(\mu\)M], BD-1063 (100 \(\mu\)M), 100 \(\mu\)M 4-((chloromethoxy)phenethyl)-N,N-dimethyl-1,1-diphenyl-1-(piperidinobutanimide (loperamide), or 100 \(\mu\)M 8-3-[p-flurobenzo[y]phenyl)-1-phenyl-1,3,8-triazaspiro(4,5)decane-4-one (spiperone). All drugs were from the Sigma Chemical except 4-IBP and BD-1063, which were from Tocris. During an experiment, a slice were submerged in oxygenated ACSF flowing at a rate of 3–4 ml/min at 35°C.

**Imaging intrinsic optical signals**

Intrinsic optical signals (IOSs) are generated by changes in light scattering or absorbance within living tissue (Andew et al. 2002). Using previously described techniques (Jarvis et al. 2001), IOSs were monitored and recorded as follows. A neocortical slice was transferred to a chamber for simultaneous imaging and electrophysiological recording. The slice was weighted at its edges with silver wire and submerged in flowing, oxygenated ACSF (3–4 ml/min). The temperature was initially 32°C and slowly raised to 35°C prior to the start of the experiment. The slice was transilluminated using a broadband, voltage regulated halogen light source (Fig. 1A) on an inverted light microscope. The light traversed a bandpass filter which transmitted red to near-infrared light (690–1,000 nm). Video frames were acquired using a COHU charge-coupled device (CCD) that was set for maximum gain and at medium black level. The gamma level was set to 1.0 so that the CCD output was linear with respect to changes in light intensity. Frames acquired at 30 Hz were averaged and digitized using a frame grabber (DT 3155; Data Translation) in a Pentium computer controlled by Axon Imaging Workbench (AIW) software (Axon Instruments, Foster City, CA).

Experiments entailed acquiring a series of images. Each image was an average of 128 or 256 images. Averaged images were saved to the hard drive and archived on recordable compact discs. An image series evoked intrinsic optical signaling over time. The first averaged image was...
in a series served as a control ($T_{cont}$), which was subtracted from each subsequent experimental image of that series ($T_{exp}$). The resulting series of subtracted images revealed changes in light transmittance (LT) over time. The change ($\Delta T$) was expressed as the digital intensity of the subtracted images ($T_{exp} - T_{cont}$) divided by the gain of the intrinsic optical signal (see formula in the following text). The gain was set using the Axon Imaging Workbench (AIW) software. The change in light transmission was visually displayed using a pseudocolour intensity scale. Zonal volumes of interest (ZOs) were selected to quantify and graphically display the experimental data and were saved for off-line analysis.

**Analysis of optical data**

Graphical and statistical analyses of data were carried out using SigmaPlot for Windows (Jandel Scientific). Images were imported and figures were prepared using CorelDraw. The data from the IOS imaging experiments were analyzed such that changes in LT of a given ZOI were expressed as percent changes of the $T_{cont}$ for that region, taken from the control image. That is

$$LT = \frac{(T_{exp} - T_{cont})/gain}{T_{cont}} \times 100 = \frac{\Delta T}{T} \%$$

This normalized the graphical data across the different regions of the neocortex, which was necessary because of the variation in opacity that caused different initial LT values ($T_{cont}$). The means were calculated and reported using a paired Student’s t-test for statistical analysis.

**Measurement of extracellular field potentials**

Extracellular recording micropipettes (10-20 MΩ) were pulled from thin-walled capillary glass. A micropipette was filled with 2 M NaCl, mounted on a three-dimensional micromanipulator and lowered into layers II/III. A silver wire coated with AgCl connected the recording micropipette to the probe of an intracellular amplification. A 5-ms pulse. The output was monitored with an on-line oscilloscope. Amplified signals were digitized and stored on video cassette tape. Several evoked field potentials were signal-averaged using a PC computer with Pclamp software (Axon Instruments).

**Results**

**Inducing recoverable and repeatable waves of SD with elevated KCl**

A transient negative shift in the voltage trace lasting about a minute represents the electrophysiological hallmark of SD. Briefly exposing the slice to 26 mM KCl for 1-2 min evoked the classic negative shift of 5- to 15-mV amplitude recorded in layers II/III (Fig. 1D, left). The signal returned to baseline within 2 min and could be evoked with repeated KCl exposure as expected for SD. The orthodromic response evoked by stimulation to layer VI was lost during each SD (not shown) but recovered within 3-4 min of SD onset (Fig. 1D, right). In the same way, SD was evoked and imaged by monitoring the associated change in light transmittance (LT). KCl exposure induced SD in 14 of the 15 slices imaged. Each slice was allowed to recover to baseline before a second and sometimes a third wave of SD was initiated in the same manner. One representative slice is here described through three consecutive SD events. An increase in LT initiated focally within layers II/III. The focus expanded concentrically and propagated along all cortical layers at 4 mm/min (Fig. 1B, left). Because the KCl was bath applied, the number of foci in a hemisected slice of neocortex varied between 1 and 3. Propagation also varied from 3 to 6 mm/min. The mean peak LT increase at the SD front was 97.5 ± 8.3% in layers II–III (Table 1) and ranged the overlying neocortex. To test slice health, the orthodromic field potential was evoked with a square pulse (0.1-ms duration; 0.25 Hz), and the intensity was adjusted to produce a near maximal response. A caliberator connected between the bath and ground generated a 1-mV, 5-ms pulse. The output was monitored with an on-line oscilloscope. Amplified signals were digitized and stored on video cassette tape. Several evoked field potentials were signal-averaged using a PC computer with Pclamp software (Axon Instruments).

**Table 1. Mean peak changes of light transmittance in layers II/III of rat neocortex**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>n</th>
<th>Mean Peak LT Increase, %</th>
<th>SD front</th>
<th>Post-SD swelling</th>
<th>Time to SD Onset, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>97.8 ± 8.3</td>
<td>124.4 ± 12.9</td>
<td>1:26 ± 32</td>
<td></td>
</tr>
<tr>
<td>GluR antagonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP-5</td>
<td>15</td>
<td>Blockaded</td>
<td>127.2 ± 13.3 (reduced)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>MK-801</td>
<td>9</td>
<td>Blockaded</td>
<td>113.7 ± 10.8 (reduced)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>CNQX</td>
<td>6</td>
<td>Blockaded</td>
<td>135.1 ± 15.6 (reduced)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>$\sigma_R$ agonists</td>
<td>5</td>
<td>93.2 ± 19.4</td>
<td>122.8 ± 8.9</td>
<td>1:29 ± 23</td>
<td></td>
</tr>
<tr>
<td>DM (1 μM)</td>
<td>5</td>
<td>104.4 ± 13.2</td>
<td>118.6 ± 15.7</td>
<td>1:19 ± 16</td>
<td></td>
</tr>
<tr>
<td>DM (10 μM)</td>
<td>7</td>
<td>Blockaded</td>
<td>95.4 ± 14.6</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>DM (100 μM)</td>
<td>13</td>
<td>Blockaded</td>
<td>7.3 ± 4.9* (reduced)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>DM + AP-5</td>
<td>5</td>
<td>Blockaded</td>
<td>12.8 ± 4.4* (reduced)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>DM + dipperone†</td>
<td>5</td>
<td>Blockaded</td>
<td>52.3 ± 16.4* (reduced)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>DM + 3-PPP</td>
<td>6</td>
<td>71.7 ± 9.8</td>
<td>106.8 ± 14.3</td>
<td>1:21 ± 26</td>
<td></td>
</tr>
<tr>
<td>DM + BD-1063</td>
<td>5</td>
<td>96.4 ± 7.0</td>
<td>115.8 ± 9.6</td>
<td>1:33 ± 12</td>
<td></td>
</tr>
<tr>
<td>CP (100 μM)</td>
<td>4</td>
<td>Blockaded</td>
<td>9.0 ± 6.4* (reduced)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>4-IBP (30 μM)</td>
<td>10</td>
<td>Blockaded</td>
<td>9.4 ± 6.1* (reduced)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>4-IBP + 3-PPP</td>
<td>5</td>
<td>88.7 ± 9.2</td>
<td>101.9 ± 13.3</td>
<td>1:24 ± 26</td>
<td></td>
</tr>
<tr>
<td>4-IBP + BD-1063</td>
<td>5</td>
<td>103.4 ± 14.1</td>
<td>115.7 ± 8.8</td>
<td>1:36 ± 18</td>
<td></td>
</tr>
<tr>
<td>$\sigma_R$ antagonists</td>
<td>6</td>
<td>92.2 ± 17.4</td>
<td>89.4 ± 12.7</td>
<td>1:38 ± 15</td>
<td></td>
</tr>
<tr>
<td>(+)-3-PPP</td>
<td>5</td>
<td>93.8 ± 9.1</td>
<td>105.8 ± 7.5</td>
<td>1:34 ± 19</td>
<td></td>
</tr>
</tbody>
</table>

Responses are to brief application of 26 mM KCl for 2 min in slices pretreated for 15 min with the listed compounds. Data reported are means ± SD. LT, light transmittance; SD, spreading depression; AP-5 2R-2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitroquinoline-2,3-(1H,4H)-dione; DM, dextromethorphan; 3-PPP, 3-(3-hydroxyphenyl)-N-(1-propyl)peridine; CP, carbetapentane; 4-IBP, N-(N-benzyl)peridine-4-yl)-4-iodobenzamide. * Significantly different from control ($P < 0.05$). † Exposure to elevated [K+]o, was 4 min.

*J Neurophysiol* • VOL 88 • NOVEMBER 2002 • www.jn.org
between 85 and 95% throughout the neocortex. Within about a minute of onset, LT values decreased to near baseline levels (6.2 ± 4%). A secondary general increase in LT then developed simultaneously across layers I–VI, with a maximum of 124 ± 13% as measured in layers II/III (Fig. 1B and C). The signal did not propagate, as evidenced by its coincident onset in several regions, and lasted about 5 min. Subsequent brief exposure to KCl induced a second and sometimes third wave of SD (Fig. 1B and C). Elevated LT again initiated in layers II/III and propagated (peak LT = 107% for the 2nd and 3rd SD). The initiation foci were in different regions of layers II/III than the sites of the first SD event. Again after the SD front passed, LT decreased to near baseline (8.3 ± 5%) before increasing to 134 ± 18%. This generalized signal increase again subsided to baseline within 5 min of its onset (Fig. 1B and C).

There was no statistical difference between the maximum LT changes associated with the first or successive SD events within a given slice or between slices (n = 14, P < 0.05). The same held true for the secondary general swelling (P < 0.05). In some slices, all regions of layer II/III apparently had equal potential to initiate and support SD as described above. Other slices however showed SD originating at the same site in layers II/III (not shown).

**Glutamate receptor (GluR) antagonists**

As in the preceding text, a second group of control slices were exposed to 26 mM KCl ACSF (Fig. 2A, left) which induced SD with a mean LT peak of 133 ± 16% (n = 10) and then a generalized LT increase averaging 137 ± 8%. Note that plotting two spatially separate regions along layers II/III shows different onset times, demonstrating SD migration. In contrast the post-SD increase in LT is temporally locked to the time of global KCl elevation. After a 10-min recovery period, 2 mM of the non-specific GluR antagonist kynurenate was bath applied for 15 min and then KCl co-applied. In all 10 slices, kynurenate blocked SD onset (Fig. 2A, right). The cortex still underwent a non-propagating and global increase in LT (mean peak LT = 135 ± 16%) before returning to a slightly elevated baseline level of 11 ± 6% (Table 1). In other words, kynurenate blocked SD but not the generalized LT increase, indicating that it was independent of SD and did not require GluR activation. We tentatively ascribed the signal to general cell swelling caused by elevated KCl in the bath.

As shown in Fig. 2B, similar pre-treatment with the specific competitive NMDAR antagonist AP-5 successfully prevented SD onset in 15 of 15 slices following 2 min of 26 mM KCl (50 µM tested in 10 slices, 100 µM tested in 5 slices). As with kynurenate, the slices displayed a non-propagating elevated in LT, again apparently as an after-effect of K⁺ exposure (mean peak = 127 ± 11%). AP-5 pretreatment prevented SD even when the KCl exposure was increased to 10 min but again failed to alter the non-propagating LT increase (n = 5). Likewise in nine of nine slices the non-competitive NMDAR antagonist MK-801 blocked SD but not the general swelling (Fig. 2C), similar to kynurenate and AP-5 (Table 1).

Pretreatment with the specific non-NMDAR antagonist CNQX (10 µM) failed to prevent or to delay SD and did not alter SD propagation in five of five slices (Fig. 2D). The initiation and propagation of SD was similar in waveform. The mean peak ΔLT at the SD front (93 ± 19%) was not statistically different from the SD control (93 ± 19%). As shown in Fig. 2A, left, KCl exposure to 10 min but againMK-801 blocked SD but wise in nine of nine slices the non-competitive NMDAR antagonist D-AP-5 successfully pre-

![FIG. 2. Pretreatment with N-methyl-D-aspartate (NMDA) receptor antagonists (but not a non-NMDA receptor antagonist) blocks SD evoked by elevated KCl. A: time course of LT change (ΔLT%) in layers II/III in response to 26 mM KCl for 1.5 min (left). Traces from 2 different ZOIs show that the SD signal propagates while the general swelling does not. Following pre-incubation of the same slice in kynurenate (2 mM) for 30 min, SD was blocked but not the general swelling (right). B: pre-incubation with the competitive NMDA receptor (NMDAR) antagonist D-2-amino-5-phosphonovaleric acid (AP-5; 50 or 100 µM) for 15 min followed by co-application with elevated KCl (26 mM) blocked SD. Only a general non-propagating increase in LT was induced. C: pre-treatment in 100 µM MK-801 for 15 min followed by co-application with elevated KCl blocked SD. The general increase in LT remained. D: pre-incubation in 10 µM 6-cyano-7-nitroquinazoline-2,3-(1H,4H)-dione (CNQX) for 15 min did not block SD or the general swelling.](http://jn.physiology.org/DownloadedFrom/10.220.33.1onMay24,2017)
cally different from control slices ($P < 0.05$, Fig. 2A, left) nor did the peak secondary swelling statistically vary from control slices ($P < 0.05$). CNQX at 10 μM effectively blocked cell swelling induced by the non-NMDAR agonists domoate or kainate (Andrew et al. 1996; Polischuk et al. 1998). So non-NMDAR antagonism did not inhibit SD, whereas NMDAR antagonist blocked SD but not the secondary swelling. Average peak LT values and SD onset times are summarized in Table 1.

**σR agonists**

**Dextromethorphan (DM).** Reports that DM can be neuroprotective prompted us to test its ability to block SD. As a control experiment, SD was induced in a slice by elevating KCl to 26 mM for 2 min (Fig. 3A, left). Following recovery, DM (100 μM) was next applied for 15 min and then 26 mM KCl co-applied. After 3-min exposure to elevated KCl, no SD developed in 13 of 13 slices (Fig. 3A, right). Moreover, 100 μM DM completely prevented the general swelling following SD (Fig. 3A, right), unlike the NMDAR antagonists described in the preceding text (Fig. 2B and C). Remarkably, 100 μM DM could block SD even when slices were exposed to K⁺ for 6 min ($n = 5$) or even 10 min ($n = 8$). Note that the secondary swelling re-appeared and became more prominent as the duration of K⁺ exposure increased (Fig. 3, B and D). When the 15 min DM pretreatment was reduced from 100 to 10 μM, SD was still blocked but the general swelling that developed post-SD was not. It was similar to control slices without DM exposure. The mean peak LT for secondary swelling was 95.4 ± 14.6%, $n = 7$ (Fig. 3C). In other slices, further reducing DM concentration to 1 μM for incubation periods of 15 min ($n = 5$), 30 min ($n = 5$), and 45 min ($n = 7$) failed to block SD or the secondary swelling (Fig. 3, C and D). Thus DM blocked SD at 10–100 μM and in addition reduced K⁺-induced swelling to some degree at concentrations above 10 μM (Fig. 3D and Table 1).

**Carbetapentane (CP).** After a 2-min exposure to elevated KCl, no SD developed in four of four slices pretreated with 100 μM CP (not shown), identical to 100 μM DM in the preceding section. Also like DM, it almost completely prevented the post-SD swelling, again unlike the NMDAR antagonists (Table 1). Longer exposures to KCl or lower CP concentrations were not tested.

**4-IBP.** The σR agonist 4-IBP was tested against SD because unlike DM, it has negligible cross reactivity at NMDAR sites (Whittemore et al. 1997). Each of 10 slices was first exposed to 26 mM KCl ACSF for 2 min, inducing recoverable SD with a mean LT peak of 101 ± 8%, followed by a return to near baseline (Fig. 4, left). Typical generalized swelling (mean LT peak = 115 ± 9%) then developed. Following a 10 min recovery period, 30 μM 4-IBP was applied for 15 min and then co-applied with 26 mM KCl for 10 min. In all 10 slices tested, 4-IBP blocked SD onset and also greatly reduced the peak of the

![Image](http://jn.physiology.org/)

**FIG. 3.** Pretreatment with dextromethorphan (DM) blocks SD. A: in the control experiment (left), KCl induced SD that recovered by 15 min. The slice was then treated with DM (100 μM) for 15 min before co-application with elevated KCl. DM blocked SD and the generalized increase in LT, even though KCl was elevated for twice the control period. B: comparison of response in a control slice with that of varied time exposures to elevated KCl in slices pre-incubated in DM (100 μM) for 15 min. DM blocked SD when exposed to 2, 6, or 10 min of KCl elevated to 26 mM. The non-propagating swelling became difficult to block as KCl exposure was increased in duration. C: a comparison of responses to elevated K⁺ for 2 min in slices pre-incubated in DM (1 or 10 μM). Note the blockade of SD in slices pre-incubated in 10 μM but not 1 μM DM. Both concentrations were too low to block the generalized swelling. D: The average peak response of general swelling in layers II/III to 26 mM KCl in slices pre-incubated for 15 min in varying concentrations of DM (1, 10, 50, and 100 μM). Error bars represent standard deviation. The concentration of DM is plotted on a log axis. General swelling increases with duration of KCl exposure and decreases as [DM] is raised.
generalized swelling to 9.4 ± 12.7% (Table 1). However, prolonging the KCl exposure caused some swelling (Fig. 4, right).

σR and NMDAR antagonism

BD-1063 and (−)-3-PPP are reported σR antagonists. Each was bath applied to slices for 15 min prior to exposure to 26 mM KCl. Unlike the σR agonists tested in the preceding text, neither inhibited either SD onset or the secondary generalized swelling (Fig. 5A and B). Neither BD-1063 (n = 5) nor (−)-3-PPP (n = 6) affected latency to SD onset, SD propagation rate, peak LT during SD, or peak LT during generalized swelling (Table 1).

FIG. 4. A 2-min exposure to KCl evokes SD and post-SD swelling (left). However in the presence of 30 μM N-(N-benzylpiperidine-4-yl)-4-iodobenzoamide (4-IBP), even a 10-min exposure to KCl only induces some general swelling but not SD. Inset: the SD signal propagates but not the signal representing general swelling. SD propagates from one zone of interest (solid line) to another (dashed line) whereas the swelling signal does not propagate.

FIG. 5. A, B: pretreatment with the sigma-one receptor antagonist 3-(3-hydroxyphenyl)-N-(1-propyl)piperidine (A) or BD-1063 (B) does not block SD induced by elevating KCl. C and D: these same antagonists, while not affecting SD themselves, remove SD block by σR agonists such as DM (C) or 4-IBP (D).
Having established that these two \( \sigma_1 \)R antagonists neither promote nor block SD, we investigated if either could inhibit SD blockade by the previously tested \( \sigma_1 \)R agonists. Application of 100 \( \mu \)M (+)-3-PPP or BD-1063 for 15 min was followed by co-application of DM \((n = 11)\) or 4-IBP \((n = 10)\) for 15 min prior to co-application of 26 mM KCl for 2 min. In all cases typical SD ensued despite the presence of the normally inhibiting \( \sigma_1 \)R agonist (Fig. 5C and D). Therefore both antagonists prevented \( \sigma_1 \)R agonist blockade of SD.

To help rule out that \( \sigma_1 \)R ligand blockade of SD is through an indirect action on an NMDAR-mediated action, slices were pretreated with 100 \( \mu \)M DM for 15 min and then exposed to NMDA (100 \( \mu \)M) for 1 min. NMDA exposure alone normally induces a non-propagating swelling, which is mediated by NMDA receptor activation in hippocampus (Andrew et al. 1996) and in neocortical gray (Jarvis et al. 2001). In the presence of DM, NMDA evoked a general increase in LT across all gray matter layers (Fig. 6A) with a maximal value of 82 \( \pm \) 21\%, statistically similar to its previously characterized response \((P < 0.05; n = 5 \text{ slices})\). Therefore DM did not reduce cell swelling evoked by activation at the NMDA receptor.

AP-5 or MK-801 prevents SD as do \( \sigma_1 \)R agonists, but only the latter reduce post-SD swelling, suggesting that this swelling is not through NMDAR activation. To confirm this, AP-5 (50 or 100 \( \mu \)M) was bath applied for 15 min, followed by co-application of 100 \( \mu \)M DM for 15 min (Fig. 6B). Then 26 mM KCl as co-applied for 2 min. As expected, elevated KCl failed to induce SD in all 5 slices but, in addition, the gener-

**FIG. 6.** DM’s effects are not mediated by NMDARs. A: response to NMDA in slices pre-incubated in DM (100 \( \mu \)M) for 15 min. A 1-min exposure to 100 \( \mu \)M NMDA at 3:00 evoked a general increase in LT throughout the gray matter. This signal did not propagate as a wave, instead developing simultaneously across the slice. B: LT values from the 2 zones of interest in A (boxes) were plotted. The plots show the time course of LT change in layers II/III of dorsolateral and dorsomedial neocortex. In a single experiment and repeated in 4 more slices, NMDA exposure induced a generalized swelling simultaneously across the entire slice. C: time course of LT change in layers II/III in response to 2 min of elevated K\(^+\) in slices pre-incubated in AP-5 and DM. Elevated K\(^+\) exposure failed to induce SD or any discernable change in LT. At 50 \( \mu \)M, AP-5 can block NMDA-induced swelling (not shown) but not that induced by KCl (Fig. 2B). It also could not reverse SD block by DM, indicating that DM does not act through NMDARs (Fig. 2B).
alized swelling was not blocked (mean peak LT = 13 ± 4%). That DM was still effective in blocking the swelling indicated that NMDARs did not mediate swelling.

**Spiperone**

Spiperone has a binding profile similar to the secondary binding associated with micromolar concentrations of several less specific σR ligands such as haloperidol, DM and (+)3-PPP. It is a D₂ dopamine antagonist that also displays α1B-adrenergic receptor antagonism. It has very low affinity for σ₁ or σ₂ receptors. Spiperone (100 μm) was bath applied for 15 min prior to co-application of 26 mM KCl for 2 min. This initially prevented SD in three of three slices, but not the secondary swelling (mean peak LT value = 94 ± 14%; Fig. 7A, dotted line). However, unlike the σ₁R agonists, when the duration of co-application of 26 mM KCl was increased to 4 min, SD was no longer blocked (n = 6, mean peak LT value = 118 ± 7; Fig. 7A, solid line). Spiperone was also tested for its potential to antagonize the effects of DM. Five neocortical slices were exposed to 100 μM spiperone for 15 min, prior to co-application with DM (100 μM) for 15 min. Then 26 mM KCl was co-applied for 6 min. In all five slices, SD blockade by DM was not antagonized by spiperone (Fig. 7B). DM was also still able to reduce generalized post-SD swelling (mean peak LT value = 52 ± 16%). Thus spiperone showed no obvious σ₁R ligand-like activity.

**Loperamide hydrochloride**

Loperamide is an antidiarrheal agent with high affinity for both peripheral and central opioid receptors. It also acts as a non-selective Ca²⁺ channel blocker of both L- (dihydropyridine sensitive) and N-type (dihydropyridine resistant, ω-conotoxin sensitive) Ca²⁺ channels in hippocampal neurons (IC₅₀ = 2.5 μM) (Thurgur and Church 1998). At higher concentrations, it acts as a weak NMDA antagonist (IC₅₀ = 73 μM), reducing Ca²⁺ influx through NMDAR-activated channels. Church and Fletcher (1995) proposed that micromolar concentrations of some σR ligands may mediate their effects through high-voltage-activated Ca²⁺ channels and showed that the effects were blocked by loperamide. We therefore tested if antagonism of Ca²⁺ channels and a weak antagonism of NMDAR channels using a high dose of loperamide could prevent SD. In all seven slices tested, a 15-min application of 100 μM loperamide prior to co-application with 26 mM KCl for 2 min did not prevent or delay the onset of SD (mean peak LT value = 111 ± 14%) as shown in Fig. 7C. The secondary general swelling was also unaltered (mean peak LT value = 129 ± 19%). Thus loperamide had no effect upon SD.

**DISCUSSION**

**Measuring intrinsic optical signals during SD**

The time course of elevated light transmittance imaged at the SD front is consistent with reversible cell swelling, which has also been measured as a transient reduction of extracellular space or increase in extracellular resistance (see review by Somjen 2001). It has been stated recently in several studies that there is no simple relation between cell swelling and LT.
(Andrew and MacVicar 1994; Andrew et al. 1997). This fits theoretically with the altered optical properties of tissue undergoing swelling. Specifically, more planar membranes, swollen organelles, reduced refractance between intra- and extracellular spaces should reduce light scatter, thereby elevating transmittance (Andrew et al. 2002). This simple relationship between cell swelling and LT is demonstrated by photon counting with the detector placed onto the surface of a brain slice, thereby avoiding a tissue/atmosphere interface (Tao 2000). As with submerged slices, osmotic swelling decreases light scatter and shrinkage increases it. Too much swelling induces SD that disrupts the signal, possibly because in this strongly hyposmotic (and unphysiological) environment, the already swollen cells may actually lose water during SD. SD measured optically in the isolated and submerged retina or lens cannot be compared to submerged brain slices because retina and lens are initially transparent and have pronounced extracellular matrices that scatter light when hydrated (Fernandes-de-Lima et al. 2001) unlike brain tissue.

Optical complexity increases if a brain slice is interfaced with the atmosphere. In this configuration (Kreisman et al. 1995; Somjen 2001), tissue swelling during SD unexpectedly increases scatter as measured by decreased LT (Snow et al. 1983; Buchheim et al. 1999; Muller and Somjen 1999) or increased reflectance (Muller and Somjen 1999; Vilagi et al. 2001). Such discontinuities are not well understood but seem to involve changes in light-scattering properties at the slice/atmosphere interface because submerged slices display a linear relation between LT and osmolality between 0 and −80 mosM and between 0 and +80 mosM (Andrew et al. 1997).

Measuring change in reflected light from a brain slice during cell swelling or shrinkage introduces further complexity. The simple explanation is that transmitted photons, by definition, have not been optically altered, undergoing only minor forward scatter. In contrast, reflected photons are scattered at larger angles, involving many more scattering events. Cells themselves scatter light at very small angles (0.5–1.5°), whereas cell organelles scatter at much larger angles (Mourant et al. 1998) so a measured LT change may have a larger component attributable to altered cell volume than reflected light. Also, scattered light is directed out of the tissue at preferred angles that can change as the tissue swells. Thus reflectance measured at a single fixed position above the tissue may undergo fluctuations as physical changes (e.g., tissue thickness, to mitochondrial volume) alter preferred scatter angle (L. Lilge, personal communication).

In the intact animal, SD is not damaging to brain tissue even when it is repeatedly evoked (Nedergaard and Hansen 1988). This is also true in brain slices interfaced with an O2-CO2 atmosphere (Aitken et al. 1988; Somjen et al. 1992) although repeated SD induction in submerged slices is more difficult. Our submerged neocortical slice preparation is able to support several SD events without damage if the superfusion rate is more than 3 ml/min. Each SD event is innocuous as evidenced by the return of both the negative shift and the IOS signal to baseline following each SD. The repeatability of the SD event as monitored optically and electrophysiologically further indicates functional health, allowing each slice to be its own control when testing if a drug blocks SD. In addition to the reversible cell swelling that accompanies the SD front, imaging transmitted light also reveals if the tissue is damaged (Obeidat and Andrew 1998; Jarvis et al. 2001). As expected no such signal followed SD in our study. Finally, the field potential could be evoked from layers II/III with little deterioration in signal before and after each SD event.

**SD blockade through σ, R mediation not NMDAR antagonism**

The cause of aura remains unknown but could involve a subtle channelopathy. The possibility of raising the SD threshold to prevent aura (and subsequent migraine pain) represents a rationale for testing drugs that might be clinically tolerable at prophylactic doses. Competitive or non-competitive NMDAR antagonists prevent SD in vivo (Hernandez-Caceres et al. 1987; Marrannes et al. 1988; Lauritzen and Hansen 1992; Nellgard and Wieloch 1992; Koroleva et al. 1998) and in interface slices (Somjen 2001). Likewise we found that the NMDAR antagonists AP-5 and MK-801 (and the general GluR antagonist kynuremate) blocked SD. It does not necessarily follow that elevated glutamate release is the cause of SD, only that NMDAR activation supports propagation (Obrenovitch 2001). As also found in vivo, we observed that AMPA receptor blockade did not affect SD. There is no consistent data pointing to a specific neurotransmitter being involved in SD initiation. Indeed Obrenovitch (2001) has argued that while reduction of Mg2+ blockade of the NMDAR-associated channel is a necessary step in the cascade of events underlying SD, elevated glutamate release actually tends to inhibit SD onset.

We tested three compounds (DM, CP, and 4-IBP) which bind to σ1 receptors and are reported agonists. Each prevented SD at 100 μM or less. CP is an antisuicide like DM but with anticonvulsant activity independent of the NMDA receptor (Apland and Braitman 1990; Leander 1989). Furthermore we found that SD block could be relieved by either one or two σ1 antagonists, BD-1063 or (+)-3-PPP. Therefore the prevention of SD appears to be through mediation by σ1 receptors.

While NMDAR antagonists block SD in vivo and in brain slices (see introduction), our results indicate that σ1, R agonists prevent SD onset independent of the NMDA receptor. Some studies show that σ1, R ligands can modulate NMDAR-mediated responses indirectly through activation of the σ receptor (Yamamoto et al. 1995), but our study indicates that SD block does not require NMDAR activation based on several findings. First, whereas many σ1, R ligands partially inhibit an NMDA receptor-mediated inward current, one exception is 4-IBP, which was “essentially inactive against all subunit combination up to its solubility limit in saline of ~30 μM” (Whittmore et al. 1997). It was also inactive at the PCP site of the NMDA receptor. Therefore the SD block by 4-IBP in our study is not acting through NMDAR antagonism. Second, unlike the NMDAR antagonist AP-5 (Andrew et al. 1996), 100 μM DM did not reduce cell swelling evoked by bath application to 100 μM NMDA. Third, the σ, R agonists tested block the general swelling that follows SD, whereas the NMDAR antagonists do not. Fourth, the σ, R ligand CP is equipotent to DM in blocking SD yet displays much less NMDAR cross reactivity than DM (Leander 1989; Apland and Braitman 1990). Fifth, we have recently shown that an SD-like response induced by simulated ischemia (the anoxic depolarization, AD) is prevented by these same σ, R agonists (Anderson et al. 2000) but not by NMDAR antagonists (Joshi and Andrew 2001; Jarvis et al. 2001).
Loperamide (Thurgr and Church 1998) and spiperone have been used to assess the specificity of suspected σR-mediated activity. The inability of loperamide to prevent SD suggests that σR-activated prevention of SD is independent of any activity at L- and N-type Ca\(^{2+}\) channels. Spiperone shares several conformational properties with σR ligands but does not bind σ₁ or σ₂ receptors (Monnet et al. 1992; Hashimoto et al. 1995). While it delayed SD onset slightly, spiperone did not prevent SD nor antagonize SD blockade by σ₁R agonists. Therefore our evidence indicates that σ₁R ligands are not blocking SD through their reported non-specific effects on NMDA receptors or Ca\(^{2+}\) channels.

**Nanomolar vs. micromolar binding by σR ligands**

Sigma receptor ligands have nanomolar binding affinities for sigma receptors (Fletcher et al. 1995; Whittemore et al. 1997; Thurgr and Church, 1998), yet SD blockade requires micromolar concentrations of DM, CP, or 4-IBP. This is in keeping with the observation that receptor-mediated signaling commonly requires more ligand than predicted from classically determined affinity constants. Numerous studies have reported σR-mediated activity in the micromolar range (Hayashi et al. 1995; Bergeron and Debonnel 1997). In intact tissue and brain slices, there is non-specific binding, non-specific uptake, and impeded penetration of ligand prior to reaching receptor sites. Moreover the initiation of signal transduction events can require more than simple receptor occupancy. For example, glutamate receptor antagonists bind at nanomolar concentrations in membrane preparations, but micromolar amounts are required to block NMDA currents (Palmer 2001) or to block excitotoxic cell swelling (Andrew et al. 1996). In fact, therapeutically useful NMDAR antagonists require micromolar amounts because only low-affinity compounds are tolerated by patients (Palmer 2001). Thus Willette et al. (1994) found that the σR ligand (+)SK&F10047 displayed a better time-course and toxicity profile in suppressing cortical SD than the high-affinity NMDAR antagonist MK-801. They ascribed (+)SK&F10047 action to a moderate affinity with the NMDA receptor, but we suggest here additional activity through σ₁R mediation.

**Post-SD generalized swelling**

Bath application of elevated KCl initiates SD consistently from one or more foci in layers II/III of the neocortex. A secondary generalized LT increase follows SD as a non-propagating event observed through cortical gray. It does not have a corresponding DC signal recorded extracellularly. Large LT increases in submerged slices invariably represent cell swelling whether evoked osmotically (Andrew et al. 1997) by glutamate receptor agonists (Andrew et al. 1996) or by elevated K\(^{+}\) (Andrew and MacVicar 1994). NMDAR antagonists block KCl-evoked SD but not the subsequent general swelling, indicating that these two events are not directly coupled. The swelling appears to be a delayed response to briefly raising [K\(^{+}\)]\(_{o}\) to 26 mM. The source of this response is conjecture: Glia act to take up and buffer elevated [K\(^{+}\)]\(_{o}\) through a passive, diffusion based mechanism (Ballanyi et al. 1987; Walz 1989). Upon SD onset, control ACSF is reintroduced and reaches the chamber within a minute. The glia may then passively release accumulated K\(^{+}\) as [K\(^{+}\)]\(_{o}\) begins to drop (Walz 1989). This rise in extracellular K\(^{+}\) occurs in a period when glia are repolarizing after the SD event, so glial K\(^{+}\) buffering capacity may be limited. The glia-mediated release might then cause the observed generalized swelling. The rise in K\(^{+}\) does not induce a second SD event because [K\(^{+}\)]\(_{o}\) does not reach a critical level (suggested to be approximately 13 mM) or because the neurons are in the refractory period lasting about 10 min between successive waves of SD (Walz 1997).

Remarkably DM, CP, or 4-IBP oppose general K\(^{+}\)-induced swelling, providing that the 26 mM KCl application is not prolonged beyond 3 min. This activity is not possessed by the two σ₁R antagonists or by loperamide, spiperone, or the GluR antagonists. Therefore σ₁R ligands may act to block SD by opposing in some way the accumulation of [K\(^{+}\)]\(_{o}\). The prevention of SD and reduction of K\(^{+}\)-induced swelling by σ₁R agonists (but not the antagonists) suggests a σ₁R-mediated mechanism that could be of potential therapeutic importance.

Video clips of spreading depression and axonic depolarization can be found at http://anatomy.queensu.ca/faculty/Andrew.cfm.

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