Latency of pupillary reflex dilation during general anesthesia

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Latency of pupillary reflex dilation during general anesthesia. J Appl Physiol 97: 725–730, 2004; 10.1152/japplphysiol.00098.2004.—Areas of insensibility produced by neuraxial anesthesia or peripheral nerve blocks can be detected during general anesthesia by failure of noxious stimulation to trigger pupillary reflex dilation. We examined the latency of pupillary reflex dilation and the effect of fentanyl on the latency of reflex dilation during anesthesia in nine volunteers. We hypothesized that the reflex was generated by slowly conducting C nociceptive fibers and would be significantly delayed if a distal dermatome (L4) was stimulated compared with a proximal dermatome (C5). We also hypothesized that fentanyl would prolong the latency and alter the shape of the reflex. After induction of general anesthesia, pupillary reflex dilation was measured with an infrared pupillometer every 5 min after stimulations of the L4 and C5 dermatomes. Fentanyl (3 μg/kg) was then given intravenously. Pupillary reflex dilation latencies were calculated by examining each individual measurement. After 3 h, naloxone (400 μg) was given intravenously; anesthesia was then discontinued. Pupillary reflex dilation had a long latency and consisted of distinct early and late phases. No differences were found between latencies of reflex dilation after stimulation of L4 and C5 dermatomes either before or after fentanyl administration. Fentanyl at high concentrations essentially eliminated pupillary reflex dilation; but over the 180-min observation period, first early and then late dilation returned. Fentanyl produced a small increase in the latency of the initial early dilation. We conclude that pupillary reflex dilation during anesthesia is not initiated by slowly conducting C fibers and that fentanyl depresses the reflex in a stereotypical manner.

PUPILLARY REFLEX DILATION (PRD) is a midbrain reflex that has been used clinically to define the extent of local anesthetic blockade during general anesthesia. Although the reflex peaks 1 min after the stimulus starts, a more prompt assessment of sensory blockade should be possible by examining the early portion of the reflex. Thus knowledge of the latency of PRD, and the factors that influence latency, is critical for a timely and reliable detection of the reflex, especially when the reflex magnitude has been attenuated by anesthetic adjuvants including opioids (14, 19).

Studies in anesthetized animals have shown that the reflex latency is ~350 ms (2, 23). The feline reflex proceeds from the nociceptor primarily via C-fiber transmission (8) to the spinal cord and then to the midbrain where norepinephrine-containing neurons are activated. These neurons then inhibit the pupilloconstrictor neurons, resulting in a passive dilation of the pupil (11).

Although the reflex in anesthetized humans is thought to be similarly expressed (13, 28), preliminary evidence has shown that the latency of PRD in anesthetized humans is remarkably long and proceeds in two distinct phases, a primary and a secondary dilation. One explanation for the long latency of the primary dilation might be that the reflex is initiated by slowly conducting nociceptive Aδ- or C fibers. The longer nerve fibers in humans compared with cats would therefore account for the differences in observed latencies. We sought to examine this theory by comparing the latencies of the primary dilation after stimulation of proximal and distal sites in human volunteers.

Nerve conduction velocities are well characterized (1, 6, 27). These data suggest that in a 175-cm-tall human, the additional delay resulting from stimulating the L4 compared with the C5 dermatome will be 700 ms for C fibers (conduction velocity 1 m/s), 90 ms for slow Aδ-fibers (conduction velocity 10 m/s), and 45 ms for fast Aδ-fibers (conduction velocity 40 m/s). We therefore examined the latency of PRD during general anesthesia as generated from proximal and distal dermatomes, before and after intravenous opioid administration. Our first hypothesis was thus that the latency of the primary dilation is several hundred milliseconds longer when the inciting stimulus is applied to the leg than to the upper arm. Reflex latency may also be prolonged when patients are given opioids, which blunt reflex dilation (17) and activation of spinal neurons by C fibers. Our second hypothesis was therefore that opioids significantly prolong the reflex latency of the primary dilation.

A secondary purpose for this study was to analyze some of the factors involved in generating the two phases of PRD. Although the primary (initial) dilation is of short duration, our laboratory has previously observed that the late secondary dilation begins after the stimulus ends and thus may represent an after discharge brought about by temporal summation ("windup") (9). Because prior studies have shown that these late neuronal discharges after noxious stimulation are highly sensitive to suppression by opioids (26, 29), our third hypothesis was that fentanyl would preferentially block the secondary dilation but leave the primary dilation essentially intact.
METHODS

With approval of the Committee on Human Research and written consent at the University of California, San Francisco, we studied nine American Society of Anesthesiologists Physical Status 1 volunteers. Exclusion criteria included body mass index >30, history of ophthalmic or neurological disease, medications other than oral contraceptives, and age <18 or >35 yr.

Protocol. General anesthesia was induced with propofol (3 mg/kg) and vecuronium bromide (0.15 mg/kg); the trachea was intubated, and the lungs were mechanically ventilated to maintain end-tidal Pco2 near 35 Torr. Anesthesia was maintained with 5% end-tidal desflurane in 50% oxygen and 50% nitrogen. Lactated Ringer solution was given at ~3 ml·kg⁻¹·h⁻¹ through a catheter in the left arm. Vecuronium was infused to provide one to two mechanical twitches in response to supramaximal electrical stimulation of the ulnar nerve at the wrist.

PRD was induced by noxious electrical stimulation at two sites. The lower site was the fourth lumbar (L₄) dermatome on the medial aspect of the right leg. The upper site was the fifth cervical (C₅) dermatome on the lateral aspect of the right shoulder. Stainless steel needle electrodes (1.5 cm long) were inserted subcutaneously 3 cm apart at each site. Stimulation started 30 min after induction of anesthesia.

Each site was stimulated for 3 s with a 100-Hz electric current (Digitest II, Neurotechnology, Dallas, TX). The intensity of this noxious stimulus is similar to that resulting from skin incision (30). The initial stimulation site was randomly assigned. The stimulating currents and altered stimulations between C₅ and L₄ every 3 min. This is a moderate dose of the opioid. We maintained the same fentanyl concentration after fentanyl administration, but they subsequently decreased exponentially. The latency of the first dilation was considered to be the integrated area during thefirst 3 s of scan. The time to at least 90% recovery of the first dilation was recorded for each volunteer. We also determined the time required until the secondary dilation could again be observed after fentanyl administration with either C₅ or L₄ stimulations.

We used two-tailed, paired t-tests to compare latency times for different-sized PRDs before fentanyl administration and between equal-sized C₅ and L₄ PRDs before and after fentanyl. Linear regression was used to compare the time to 90% recovery of the first dilation and the time until the secondary dilation could be observed. All data reported as means ± SD unless otherwise indicated; P < 0.05 was considered statistically significant.

RESULTS

Participants were 27 ± 5.4 yr old, weighed 69 ± 8 kg, and were 176 ± 11 cm tall. There were six men and three women. Stimulating currents set before fentanyl administration were 61 ± 14 mA for the C₅ dermatome and 62 ± 9 mA for the L₄ dermatome.

Before fentanyl administration, pupillary responses were virtually identical after noxious stimulation at the L₄ and C₅ dermatomes (Fig. 1). Analysis of this curve indicated that PRD during general anesthesia has a long latency and two distinct dilations, one beginning from 700 to 1,000 ms after stimulation was started and another beginning from 3.0 to 5.0 s after the stimulus. The magnitude of both the primary and secondary dilations was inversely related to latency (Table 1).

After fentanyl administration, PRD was briefly abolished in all subjects, and the time required for this reflex to return was variable but averaged 13.3 ± 9.4 min. Serum fentanyl concentrations increased by 4.5 ± 0.9 ng/ml at 5 min after the fentanyl bolus, but they subsequently decreased exponentially. The fentanyl concentration required to inhibit PRD was characterized by a rectangular hyperbolic relationship that is typical of simple agonist-to-receptor-site interaction (Fig. 2).

Return of the pupillary dilation reflex followed a distinct pattern in all subjects: the first dilation gradually appearing within the first 30 min, and the second dilation appearing only after the primary dilation had returned to at least 90% of its prefentanyl magnitude (Fig. 3). Analysis of this curve indicated that return of the secondary dilation varied from 30 to 150 min, with the average being 69 ± 41 min. There was an excellent correlation between the 90% return of the primary dilation and reappearance of the secondary dilation after fentanyl administration (Fig. 4).

Fentanyl prolonged the latency of the primary dilation, an effect that diminished with time and was reversed by naloxone (Table 1). The latency of the secondary dilation was diminished after fentanyl administration, but this effect was not reversed by naloxone (Table 1).
DISCUSSION

Our previous experience with PRD during general anesthesia indicated that a potent noxious stimulus is required for expression of the reflex during the first 3 h of general anesthesia. For example, firm skin pressure, visible light, manipulation of the limbs, sound, and low-frequency electrical stimulation (1 Hz or below) all fail to dilate the pupil during general anesthesia with volatile anesthetics at concentrations exceeding 60% of a standard dose (i.e., 1 minimum alveolar concentration) (13, 14, 17, 21). Similarly, observation of the pupil during surgery affirms that painful stimuli are required to dilate the pupil except during emergence, profound hypotension, and/or light anesthesia (7, 13–16).

This study (13) examined the first 8 s of PRD and confirmed earlier impressions that the reflex is composed of an initial dilation with a long latency of ~800 ms and a secondary accelerated dilation that begins ~4 s after the onset of the stimulus. Our first hypothesis was that PRD, thought to be mediated by C fibers, would be delayed by several hundred milliseconds when noxious stimulation was applied distally. However, onset of dilation in response to stimulation at the distal (L₄) dermatome was virtually identical to that resulting from stimulation of the proximal C₅ dermatome. These data thus negate our hypothesis that slow-conducting C fibers account for the prolonged latency in anesthetized humans.

Our data instead suggest that onset of PRD is initiated primarily by activation of nociceptors served by the faster conducting AΔ-nerve fibers. The potent noxious stimulus we used either did not activate C fibers or the arrival of signals carried by C fibers to supraspinal sites did not alter the shape of PRD. One interpretation is that slower conducting nociceptive fibers fail to alter PRD because brain regions already activated by AΔ-fiber signals were refractory to additional excitation. This theory is consistent with the observation that C-fiber-mediated cortical activity representing nociception is difficult or impossible to observe unless conduction within the AΔ-fibers is first blocked (6, 10).

PRD during anesthesia is thought to be mediated by a neuronal pathway that extends from cutaneous nociceptors to the midbrain where inhibitory neurons are activated to depress activity in the pupilloconstrictor nucleus (11, 23). This circuit describes the reflex in cats and rats where the inhibitory

Table 1. Pupillary dilation latencies

<table>
<thead>
<tr>
<th>Dermatome</th>
<th>Pupillary Reflex Dilation, mm·s⁻¹</th>
<th>Primary Latency, ms</th>
<th>Secondary Latency, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C₅</td>
<td>L₄</td>
<td>C₅</td>
</tr>
<tr>
<td>Before fentanyl (51 ± 8 mA)</td>
<td>9.4±3.6</td>
<td>950±130</td>
<td>4.3±0.5</td>
</tr>
<tr>
<td>Before fentanyl (65 ± 6 mA)</td>
<td>16±3.6*</td>
<td>810±140*</td>
<td>3.8±0.4*</td>
</tr>
<tr>
<td>Before fentanyl (62 mA)</td>
<td>14±1.7</td>
<td>840±120</td>
<td>4.2±0.4</td>
</tr>
<tr>
<td>Just after fentanyl (62 mA)</td>
<td>7.0±1.0*</td>
<td>1,020±320†</td>
<td>4.2±0.3</td>
</tr>
<tr>
<td>Long after fentanyl (62 mA)</td>
<td>11.3±1.9†</td>
<td>960±110†</td>
<td>No dilation</td>
</tr>
<tr>
<td>Postnaloxone (62 mA)</td>
<td>14.7±1.8</td>
<td>840±230</td>
<td>No dilation</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.05, high-current (65 mA) vs. low-current (51 mA) stimulations before fentanyl. †P < 0.05 compared with before fentanyl. There were no differences in latencies between C₅ and L₄ at any time of the study.

Fig. 1. Changes in pupil diameter for 8 s after 3-s tetanic stimulation of C₅ and L₄ dermatomes before administration of fentanyl were virtually identical. Scans are averaged from all 9 volunteers. Integrated area under the baseline was 14.0 mm·s for the C₅ dermatome and 13.9 mm·s for the L₄ dermatome. Solid line, L₄; dotted line, C₅.

Fig. 2. Dose-response curve of fentanyl effect vs. mean serum concentration of fentanyl. Values are means ± SD of averages from 9 volunteers. Maximum effect was total ablation of pupillary reflex dilation of 14 mm/s.
transmitter is norepinephrine acting via an α2-adrenergic receptor on the pupilloconstrictor neurons. With Aδ-fibers conducting at 20–40 m/s and the spinothalamic tract conducting at 10 m/s, the signal should reach the midbrain in no more than 100 ms after the onset of the stimulus in humans. There is then an additional 250-ms delay at the neuromuscular junction. From our data, it is thus apparent that the remaining 450 ms of reflex latency in humans remains unexplained.

One possibility is that the reflex is not simply a two- or three-neuronal reflex, as in the cat, but is instead a multicircuit pathway that results in a long latency reflex at the iris musculature. This theory is consistent with studies demonstrating that noxious heat-induced activation of Aδ-fibers in humans provokes cortical potentials. These potentials reach a maximum at ~300 ms after stimulus onset (10). Such a pathway through the cortex and then back to the midbrain and oculomotor nerve would easily account for the long latency of PRD.

Our second hypothesis that fentanyl would specifically prolong the latency of the primary dilation was confirmed, but the magnitude of the prolongation was clinically irrelevant. Opioids are often administered during general anesthesia, but a mere 200-ms increase in the latency of PRD would not appreciably alter the ability to detect the presence or absence of PRD during combined epidural-general anesthesia. The effect of fentanyl on latency is most likely due to its depressant effect on the magnitude of PRD. Before the administration of fentanyl, we observed that weak stimulations that produced smaller PRDs resulted in slightly prolonged latencies.

Our third hypothesis, that fentanyl would eliminate the secondary dilation but leave the primary dilation intact, was confirmed. The secondary dilation therefore is consistent with some of the characteristics of windup insofar as it is preferentially depressed by opioids and outlasts the stimulus by several minutes. However, it differs from windup in not being mediated by slow-conducting C fibers, a conclusion based on the fact that there was no difference in the latency or shape of the secondary dilation after stimulations at the L4 and C5 dermatomes. Because windup has been exclusively associated with activation of C fibers (9), we conclude that, although our hypothesis was proven true, our reasoning was faulty and other mechanisms must account for the secondary dilation, which we observed. Similarly, the secondary dilation cannot be a neurophysiological representation of “secondary pain” mediated by C fibers (22, 25) as opposed to “primary pain” mediated by Aδ-fibers.

We conclude that the origin of the secondary dilation remains unclear. Most studies on windup and long-term potentiation after noxious stimulation have been performed on the wide-dynamic-range neurons of the dorsal horn (9, 29). It may be that theories based on the behavior of spinal neurons are inadequate to explain the dynamic properties of this reflex and that further investigations should consider the neuronal firing patterns of more rostral brain regions. One theory is that the secondary dilation may involve Aδ-fiber-induced burst firing of cellular groups within the rostral nociceptive circuit. We observed that the secondary dilation does not appear until the primary dilation has returned to at least 90% of the prefentanyl value. This suggests that, when a certain threshold of nociception is reached, then the secondary dilation is triggered. As demonstrated in the present study, this secondary dilation is highly sensitive to the depressant effects of opioids.

Nearly 50 years ago, Loewenfeld presented an analysis of PRD in the anesthetized cat and speculated that the hormonal delivery of norepinephrine and epinephrine to the eye after intense noxious stimulation would produce a secondary dilation in PRD by acting on the dilator muscle of the iris.
musculature (23, 24). There are several reasons why this cannot explain our data on secondary dilation. First, an onset of 4 s is too brief a time for hormonal activity to appear. Second, our laboratory has previously demonstrated that α2-adrenergic-blocking agents do not suppress the prolonged pupillary dilations after noxious stimulations in anesthetized humans (20). Finally, it is known that, although the feline iris is exquisitely sensitive to circulating epinephrine and norepinephrine, the primate iris is relatively insensitive to the hormonal effects of these agents (4).

Our observations have practical value when PRD is used to assess the extent of sensory block during general anesthesia (17). First, it clearly does not make any difference whether a proximal or distal dermatome is stimulated. The method can thus be used perfectly well to evaluate integrity of a distal peripheral nerve block. Because the clinical goal is to determine the presence or absence of the reflex rather than reflex magnitude, there is no need to stimulate longer than the latency to the first dilation; a noxious stimulus lasting only 1 s is thus sufficient, even after fentanyl administration. Second, our results indicate that the primary dilation can be readily observed even after the secondary dilation has been obliterated by fentanyl administration. Portable infrared pupilometers are now available that can readily detect the 0.2- to 0.4-mm dilations observed in the primary dilation. By avoiding high currents and long stimuli (18) the prolonged secondary dilation will not be triggered, allowing more dermatomes to be tested in a short period of time.

Limitations of our study are that we evaluated only a moderate dose of fentanyl and enrolled only young volunteers. Older patients may be more sensitive to opioids, and larger doses of opioids in all age groups would obliterate the primary dilation. By avoiding high currents and long stimuli (18) the prolonged secondary dilation will not be triggered, allowing more dermatomes to be tested in a short period of time. Older patients may be more sensitive to opioids, and larger doses of opioids in all age groups would obliterate the primary dilation. By avoiding high currents and long stimuli (18) the prolonged secondary dilation will not be triggered, allowing more dermatomes to be tested in a short period of time.

In conclusion, we studied the latency and shape of PRD after stimulating proximal and distal dermatomes. There were no differences in the latency of the primary and secondary dilations at the two sites, indicating that the reflex is not mediated by slowly conducting nociceptive C fibers. PRD was briefly obliterated by a fentanyl bolus, which subsequently produced minor changes in latency. Our data support the use of short tetanic stimuli to detect the presence or absence of sensory block during general anesthesia.

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