**Pronociceptive Actions of Isoflurane**

A Protective Role for Estrogen

Pamela Flood, M.D.,* Danette Daniel, M.B.B.S.†

**Background:** Low concentrations of inhaled anesthetics present in the early postoperative period can increase pain sensitivity. Changes in pain threshold associated with inhaled anesthetics have been reported in male mice, rats, and humans.

**Methods:** The authors compared the pain-enhancing effects of isoflurane in male and female mice in response to a thermal stimulus and studied the consequences of hormonal manipulation.

**Results:** Isoflurane produced a larger increase in pain sensitivity in female mice. Both castration and oophorectomy resulted in an increase in baseline pain sensitivity and potentiated pain enhancement by isoflurane. At stages of the estrus cycle when estrogen was low, female mice showed greater pain enhancement from isoflurane than at high estrogen stages. Treatment with exogenous estrogen reduced isoflurane-induced pain sensitivity. Exogenous testosterone treatment had a similar effect, which did not occur when enzymatic conversion to estrogen was prevented.

**Conclusions:** Because both estrogen and testosterone reduce the pronociceptive action of isoflurane, intact females may exhibit a larger increase in pain sensitivity because of their cyclical estrogen levels. Testosterone is effective in the male because of conversion to estrogen. Enhanced pain sensitivity is clearly undesirable in the postoperative setting. If these findings also apply to humans, the menstrual cycle may be an important factor in determining pain levels after emergence from general anesthesia.

INCREASED pain sensitivity after anesthesia was first documented in a population of women having gynecologic surgery in 1960.¹ In the same study, this phenomenon was mimicked in male and female volunteers while inhaling subanesthetic concentrations of the implicated drug. Although the pain-enhancing effects of general anesthetics have long been known, clinical research in this area has languished and most clinicians are unaware of this paradoxical action. More recently the pain-enhancing effects of inhaled anesthetics have been demonstrated in animal models using male rats²,³ and female mice,⁴ but the genders have not been directly compared. Studies in animal models have suggested the involvement of noradrenergic neurons in the pronociceptive actions of isoflurane⁵ and modulation by nicotinic acetylcholine receptors.⁴ Gender differences have been manifest in both systems.⁵,⁶

Our preliminary experiments suggested that female mice had greater pain enhancement than male mice from low isoflurane concentrations. To determine whether the variation that we found was due to differences in sex hormones, we studied the animal’s pain response in the presence of isoflurane under different hormonal conditions.

**Materials and Methods**

**Animals**

Male and female 129J mice (Jackson Laboratories, Bar Harbor, ME) at 6 to 10 weeks of age were used. All procedures were approved by the Columbia University Animal Care Committee. Animals underwent oophorectomy (OVX) or castration according to standard techniques at least 2 weeks before experimentation. Estrogen withdrawal was determined in females when the vaginal smear showed leukocytes on consecutive days after oophorectomy.

**Estrus Stage Determination**

Twenty intact female mice were examined for their estrus stage before behavioral testing. A slide was prepared from a vaginal saline aspirate and the mouse staged according to standard criteria.⁷ Staging was confirmed by an investigator blinded to the behavioral data. Each mouse was tested not more than once in 2 days, until they had been tested in each stage of estrus.

**Behavior**

We measured hind paw withdrawal latency in up to five unrestrained mice (per study) housed individually in clear plastic chambers. The chambers rested on a clear glass plate. Over the chambers we placed a clear Plexiglas enclosure that rested on a silicone rubber gasket that produced a seal to the glass plate. Gas-tight fittings at either end permitted delivery to and scavenging of isoflurane. Isoflurane (Abbott Laboratories, North Chicago, IL) in oxygen was delivered from a variable-bypass vaporizer. Concentrations of isoflurane were monitored with an infrared analyzer (RGM; Datex-Ohmeda, Madison, WI) calibrated with gas chromatography. The animals equilibrated at each isoflurane concentration for at least 15 min before testing. The glass plate was warmed to minimize body heat loss. To diminish exploratory...
activity, the mice were acclimated to this environment for at least 30 min before commencing the study. After acclimation, a movable source of radiant heat was applied from a lamp through an aperture under the glass plate to the hind paw of the resting mouse. The testing stimulus was 15% of maximal and caused an average increase to 38°C at movement under control conditions. An investigator who was blinded to the treatment group measured the latency from the onset of the application of the stimulus (heat) to the time the mouse moved its hind limb. Animals were used more than once for isoflurane testing with at least 2 days between testing. Intact females were tested at each stage with isoflurane of estrus. The same mouse was tested with isoflurane both before and after oophorectomy or castration.

**Drug Treatment**

Estrogen (17 β-estradiol, Sigma-Aldrich Chemical, St. Louis, MI) was given in sesame oil *via* intraperitoneal injection, 3 h before behavioral testing. Testosterone decanoate (Sigma-Aldrich) was injected intraperitoneally 4 h before experimentation. Steroid hormones were administered as described in previous pain studies. An inhibitor of the aromatase enzyme that catalyzes the conversion of testosterone to estrogen (Sigma-Aldrich) was injected the evening before and 1 h before testosterone injection. After isoflurane testing with steroid treatment, the animals were killed.

**Statistics Analysis**

The concentration response relationship in figure 1 suggests both nociceptive and antinociceptive activity for isoflurane, separable by concentration. We compared the effect of a concentration in the pronociceptive range (0.25%), under different hormonal treatment conditions. The percentage change in withdrawal latency is compared under different treatment conditions with a *t* test modified with the method of Bonferroni. When the same animal is compared with different treatments a paired *t* test is used and when different animals are compared an unpaired *t* test is used. *P* < 0.05 is considered significant.

**Results**

Intact female mice had significantly shorter withdrawal latency while breathing isoflurane concentrations up to 0.4% (fig. 1, A). Male mice did not demonstrate a significant pronociceptive response to isoflurane under these same conditions (fig. 1, B). These mice were anesthetized by approximately 1.4% isoflurane and this value did not differ between males and females. After castration or O VX, pain threshold increased significantly in both genders with an associated enhancement in the pronociceptive effects of isoflurane (fig. 1).

![Image](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931207/)

**Fig. 1.** Effect of isoflurane on intact and castrated mice. (A) Hind paw withdrawal latency (HPWL) is shown for intact female mice (●) treated with subanesthetic concentrations of isoflurane from 0 to 0.75%. Isoflurane causes a biphasic change in pain sensitivity with increased sensitivity (18%) induced by concentrations up to 0.38 volume % and antinociception at higher concentrations. These mice are immobilized by isoflurane 1.4%. The mice become more sensitive to the heat stimulus after oophorectomy (○); *t* test; "*P* < 0.01, baseline comparison between intact and after oophorectomy). The pronociceptive action of isoflurane is enhanced after oophorectomy (20% *t* test; "*P* < 0.001, oophorectomy baseline latency compared to maximal hyperalgesia), but the analgesic action of isoflurane is unchanged. Twenty mice were studied intact, and 18 of those mice were studied after oophorectomy. (B) Intact male mice (■) do not have a significant pronociceptive response to isoflurane. After castration (□), baseline pain sensitivity is enhanced (*t* test; "*P* < 0.001, intact and castrated baseline latency compared) and a pronociceptive phase unmasked (10% decrease; *t* test; "*P* < 0.001, castrated baseline latency compared with maximal). Ten male mice were studied both intact and after castration.

We then compared the mouse’s pronociceptive response elicited by 0.25% isoflurane across the estrus cycle. We found that at stages associated with high estrogen levels (2, 3, and 5) the pronociceptive effect of isoflurane is reduced. Conversely, at estrus stages associated with low estrogen levels isoflurane pronociception was maximal and was not different from that after oophorectomy (fig. 2, A). Treatment with 17β-estradiol in OVX mice counteracted the pronociceptive response to isoflurane in a dose-dependent manner (fig. 2, B). Although baseline withdrawal levels were lower at the estrus stages associated with the highest estrogen levels, the range was small (12–14 s) and the differences were not significant.

In castrated males, isoflurane pronociception was enhanced. We tested the effect of testosterone on isoflurane pronociception in OVX mice. Testosterone, like estrogen, reduced the pronociceptive effect of isoflurane (fig. 3, A). There was no systematic effect of testosterone on baseline withdrawal latency. Because testosterone can be converted to estrogen with the enzyme aromatase, the mice were treated with an aromatase inhibitor prior to testosterone treatment. Testosterone
no longer prevented the pronociceptive action of isoflurane in the presence of an aromatase inhibitor. As a result, we concluded that the protective effect of testosterone in females was dependent on conversion to estrogen.

Under our testing conditions, intact males did not have a pronociceptive response to 0.25% isoflurane. However, when endogenous testosterone conversion to estrogen was prevented by treatment with an aromatase inhibitor, the animals did exhibit an increase in pain sensitivity to isoflurane (fig. 3, B). These findings suggest that estrogen treatment induces a physiologic condition that is resistant to isoflurane pronociception. This hormonal setting occurs in a cyclic manner in the female and through conversion of testosterone to estrogen in the male.

Discussion

This is the first study to compare the pronociceptive properties of isoflurane in male and female mice. The foregoing data suggest that both testosterone and estrogen induce a physiologic state resistant to the pronociceptive effects of isoflurane. When either ovarian or testicular hormones are removed, baseline pain threshold increases suggesting the existence of a tonic inhibitory influence that is reduced by estrogen and testosterone. Isoflurane reduces this tonic inhibition with a peak action at 0.38%. Intact females have a much greater pronociceptive response to isoflurane than males and are more sensitive to the pronociceptive effects of residual anesthetic at low estrogen phases of their cycle. Presumably, females may show a greater pronociceptive
response to isoflurane than males, due to cyclic variations in hormonal levels, whereas those of males are relatively stable over time. The greater pronociceptive response to isoflurane in females at low estrogen phases of the cycle and after oophorectomy could be due to low estrogen levels or estrogen withdrawal. Males in contrast have constant testosterone concentrations that are converted to estrogen.

The effect of testosterone on isoflurane pronociception is apparently dependent on conversion to estrogen. The role of estrogen in the male has been enigmatic until recently male estrogen receptor knockout mice have suggested that estrogen plays a role in sexual behavior and aggression. In our studies, estrogen, converted from testosterone in males, induces a state resistant to isoflurane pronociception.

Recent evidence has implicated both the α-adrenergic and neuronal nicotinic receptor systems in the pronociceptive actions of isoflurane and hormonal modulation of both systems have been described. Descending inhibitory noradrenergic tone is unregulated by estrogen and progesterone. Noradrenergic axons from cells in the midbrain express nicotinic receptors on their spinal terminals that when activated, enhance the release of norepinephrine. In intact rats, nicotine increases the release of norepinephrine in the spinal cord. Isoflurane inhibits nicotinic receptors at these low concentrations, whereas its action on other known targets is below threshold. By inhibiting tonically active presynaptic neuronal nicotinic receptors, isoflurane could decrease the release of norepinephrine, remove a tonic analgesic input and thus increase pain sensitivity.

In addition to its many genomic effects, estrogen can act as a direct inhibitor of nicotinic activation. In a high estrogen state, nicotinic control of norepinephrine release could already be inactivated and thus isoflurane would have no effect. The lower baseline withdrawal latency in intact compared to OVX or castrated animals would be in accord with this possibility. However, acute treatment with exogenous estrogen did not reduce baseline withdrawal latency and there were no statistically significant changes in baseline withdrawal latency over the estrus cycle. These findings suggest that the affects of estrogen on baseline withdrawal latency may require chronic rather than acute treatment. In contrast, acute estrogen treatment did have a significant affect on the pronociceptive actions of isoflurane (fig. 2).

The low concentrations of isoflurane that we studied may be present on emergence from a general anesthetic. If our results in mice hold true in people, the hormonal status of a patient may interact significantly with residual anesthetic to influence postoperative pain sensitivity.

References

2. Zhang Y, Eger EI II, Dutton RC, Sonner JM. Inhaled anesthetics have hyperalgesic effects at 0.1 minimum alveolar anesthetic concentration. Anesth Analg 2000; 91:462–6