INTRAPLANTAR HYPERTONIC SALINE, A RAPID, SENSITIVE, AND REPEATABLE ASSAY FOR ANALGESICS IN MICE

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INTRODUCTION / AIM

Numerous animal pain assays have evolved to evaluate analgesics, highlighting the complexity of modeling pain and analgesia. These are constantly modified to improve their validity and predictableness. However, they remain insensitive sensitive at predicting potential analgesics and further research and assay development is needed. Hypertonic saline has been used an allogenic agent particularly in humans where it has been used to induce muscle or joint pain. A few studies have adapted hypertonic saline for the induction of pain in mice. Hwang AS et al demonstrated that intradermal injection of hypertonic saline at the lower abdomen of a mouse produced short-lived assessable nociceptive behaviors, which were blocked by opioids. Alessandri-Haber N and colleagues showed the intraplantar injection of hypertonic saline produced quantifiable licking, and flinching lasting up to 5 min. They did not study whether known analgesics block nociception induced by hypertonic saline. Here, we have determined the nature of the responses to intraplantar hypertonic saline, including the variability, and time course. This background was used to determine the ability to detect a wide range of analgesics, the time course of the nociceptive behaviors, and to assess repeated injection of hypertonic saline as a way to refine number of mice used.

METHODS

All procedures were executed in randomized blinded fashion. Video-recorded experiments were analyzed by a blinded observer. Adult female CD-1 mice were used one-week post arrival to the animal facilities. On test day, mice were habituated to test environment for 2 hours. To determine the optimal concentration of saline mice were given local intraplantar injection of saline (0.9, 2, 5, and 10%) and from the video record the concentration-response curves and time courses were calculated. For testing sensitivity to analgesic drugs morphine (1, 3, 7, 10 mg/kg i.p.), ASA (100, 300 mg/kg i.p.), and lidocaine (100 µg i.pl co-injected with 10% saline). Fifteen to thirty minutes after morphine or ASA 10 microliters of 10% saline was injected. The mice were placed in the habituation chamber and video recorded for one hour. To test the feasibility of repeated testing in a mouse, a second injection at same site was made after 4 hours or after 7 days. Multiple injections (four) were made 5 days apart. Time spent licking and flinching of the injected paw in the first five minute was used in analyzing results.

RESULTS

In line with previous studies, intraplantar hypertonic saline produced intense licking and flinching in a concentration dependent fashion with 10% producing the most robust and less variable results. Therefore 10% was to test analgesia and to evaluate repeated testing in the same

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mouse. Morphine dose dependently (IC50 = 3.6 mg/kg) reduced licking induced by intraplantar 10% saline. 300 mg/kg dose of aspirin significantly reduced liking time. Lidocaine (1%) significantly attenuated but did not produce complete blockade of the nociceptive responses. Behavioral responses induced by intraplantar 10% saline were not altered when repeated either a second time (4 hours or 7 day later) or multiple times (4 injections 5 days intervals).

**DISCUSSION / CONCLUSIONS**

This study demonstrated that intraplantar hypertonic saline can be used as a pain assay that is consistent, has low variability, and is sensitive to standard analgesics. As it can be repeated in one animal the variability is low and fewer mice are required than when each mouse is used once. One of the requirements of predictive pain assay is its ability to detect wide range of analgesic (Le Bars,2000). This assay is sensitive enough to detect not only opioids but also a weak analgesic like aspirin. The repeated use of this assay does demonstrate learning as the responses remained unchanged. In conclusion, intraplantar 10% hypertonic saline is rapid assay with low variability that detects a wide range of analgesics. We recommend that this assay be considered a standard analgesic assay.

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