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Effects of Monoamine Reuptake Inhibitors in Assays of Acute Pain-Stimulated and Pain-Depressed Behavior in Rats

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Abstract: Pain is associated with stimulation of some behaviors (eg, withdrawal reflexes) but depression of many other behaviors (eg, feeding, locomotion, positively reinforced operant behavior). Drugs that block reuptake of serotonin, norepinephrine, and/or dopamine are widely used to treat depression, and they have also emerged as useful drugs for treatment of pain. This study compared effects of selective and mixed-action inhibitors of serotonin, norepinephrine, and/or dopamine reuptake in assays of acute pain-stimulated and pain-depressed behavior. Intraperitoneal injection of dilute acid served as a noxious stimulus to stimulate a writhing response or depress intracranial self-stimulation (ICSS) in Sprague Dawley rats. Selective reuptake inhibitors of serotonin (citalopram, clomipramine) and norepinephrine (nisoxetine, nortriptyline) and a mixed-action reuptake inhibitor of serotonin and norepinephrine (milnacipran) blocked acid-stimulated writhing but failed to block acid-induced depression of ICSS. Selective dopamine reuptake inhibitors (RTI-113 [3ß-(4-chlorophenyl)tropane-2ß-carboxylic acid phenyl ester hydrochloride], bupropion) and a triple reuptake inhibitor of dopamine, serotonin, and norepinephrine (RTI-112 [3ß-(3-methyl-4-chlorophenyl) tropane-2ß-carboxylic acid methyl ester hydrochloride]) blocked both acid-stimulated writhing and acid-induced depression of ICSS, although these drugs also produced an abuse-related facilitation of ICSS in the absence of the noxious stimulus. These results support further consideration of dopamine reuptake inhibitors as candidate analgesics, although abuse liability remains a concern.

***Perspective:** Monoamine reuptake inhibitors are used to treat depression and some forms of pain. This study examined effects of monoamine reuptake inhibitors in a preclinical assay of pain-related behavioral depression. The results support further consideration of dopamine reuptake inhibitors as candidate analgesics under selected circumstances, although abuse liability remains a concern.*

***Key words:** Analgesia, antidepressant, dopamine, intracranial self-stimulation, monoamine reuptake inhibitor.*

Monoamine reuptake inhibitors are drugs that block transporters for the monoamine neurotransmitters serotonin, norepinephrine, and/or dopamine.[30] These drugs have a long history of use as antidepressants, and more recently, monoamine reuptake inhibitors have emerged as medications for the treatment pain.[27,42,60,64,71] This latter application of monoamine reuptake inhibitors is rooted in both the neurobiology of nociception and the symptomology of pain. With regard to neurobiology, bulbospinal monoaminergic pathways have well-established roles in descending modulation of nociceptive input from primary to secondary nociceptors in the spinal dorsal horn, and serotonergic and noradrenergic pathways play primarily an inhibitory role in spinal nociceptive processing.[21,59] Supraspinal monoaminergic pathways have also been implicated in preclinical measures of nociception and clinical measures of pain, and in particular, data from multiple sources suggest a role for mesocorticolimbic dopaminergic systems in the subjective experience and behavioral expression of pain.[1,9,65,73] With regard to symptomology, there is high comorbidity between pain and depression.[4,22,29] In particular, pain is often associated with a depression of behavior, and this pain-related depression of behavior can serve as a diagnostic indicator of pain and a target of

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pain treatment in both veterinary and human medicine.[18,47,48] Taken together, these considerations have suggested that monoamine reuptake inhibitors with established antidepressant activity may have utility in treating pain, and especially the depression-related aspects of pain.

The objective of the present study was to systematically evaluate effects of monoamine reuptake inhibitors in complementary assays of acute pain-stimulated and pain-depressed behaviors that have been used previously to examine opioid, cannabinoid, and nonsteroidal anti-inflammatory drugs.[35,49-51,58] Specifically, intraperitoneal injection of dilute acid was used as an acute chemical noxious stimulus in rats to stimulate a writhing response (also called a "stretching" response) and to depress intracranial self-stimulation (ICSS). Abdominal writhing is a commonly used dependent measure of nociception in assays of pain-stimulated behavior using intraperitoneal administration of dilute acid or other chemical irritants as the noxious stimulus, and antinociception is indicated by reduction in writhing.[28,46,52] ICSS, by contrast, is commonly used to assess changes in motivated behavior and affect in experimental subjects,[11,68] but it can also be used to evaluate effects of noxious stimuli and candidate analgesics. For example, ICSS promotes high levels of stable responding that can be depressed by intraperitoneal acid administration, and antinociception is indicated by a blockade of acid-induced depression of ICSS.[48] The rationale for studying behavioral responses to an acute noxious stimulus was 2-fold. First, this study was intended to serve as the first step in a broader investigation on effects of monoamine reuptake inhibitors and other drugs on behavioral depression associated with acute and chronic pain states. Second, the most salient discrepancies between preclinical and clinical research on analgesic effects of monoamine reuptake inhibitors have occurred in studies of acute pain. For example, although preclinical studies often report apparent antinociceptive effects of norepinephrine and/or serotonin reuptake inhibitors in assays of acute pain,[3,8,56,62] clinical studies typically show little or no analgesic efficacy of these compounds in assays of acute experimental or clinical pain,[19,20,23,69] and these monoamine reuptake inhibitors are not indicated for treatment of acute pain. Assays of acute acid-stimulated and acid-depressed behavior were intended to further elucidate this discrepancy in the literature. We hypothesized that monoamine uptake inhibitors would block acid-stimulated writhing but would be less effective in assays of acid-depressed ICSS.

Methods

Subjects

A total of 98 male Sprague Dawley rats (Harlan, Frederick, MD) weighing 297 to 334 g at the time of surgery were used for the studies of lactic acid-stimulated writhing (n = 46) and ICSS (n = 52). Rats were housed individually and were maintained on a 12-hour light/dark cycle with lights on from 6:00 a.m. to 6:00 p.m. Rats had free access to food and water except during testing. Animal maintenance and research were in compliance with National Institutes of Health guidelines on care and use of animal subjects in research, and all animal use protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

ICSS

Surgery

All rats were implanted with a bipolar stainless steel electrode (Plastics One, Roanoke, VA) using stereotaxic surgery. Each bipolar electrode consisted of a cathode (.25 mm in diameter and covered with polyamide insulation except at the flattened tip) and an anode (.125 mm in diameter and uninsulated). During surgery, rats were anesthetized with isoflurane gas (2.5–3% in oxygen; Webster Veterinary, Phoenix, AZ). The cathode was implanted in the left medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral from midsagittal suture, and 7.8 mm below dura). The anode was wrapped around 1 of 3 skull screws to ground the implant, and the skull screws and electrode were affixed to the skull with orthodontic resin. Rats received 5 mg/kg ketoprofen as a postoperative analgesic immediately after and 24 hours after surgery, and rats recovered for at least 7 days postsurgery prior to commencing ICSS training.

Apparatus

ICSS experiments were conducted in sound-attenuating boxes that contained modular acrylic test chambers (29.2 × 30.5 × 24.1 cm) equipped with a response lever (4.5 cm wide, extended 2.0 cm through the center of 1 wall, 3 cm off the floor), stimulus lights (3 lights colored red, yellow, and green positioned 7.6 cm directly above the response lever), a 2-W white house light, and an ICSS stimulator (Med Associates, St. Albans, VT). Electrodes were connected to the stimulator via a swivel connector (Model SL2C; Plastics One, Roanoke, VA). The stimulator was controlled by computer software that also controlled all programming parameters and data collection (Med Associates).

Behavioral Procedure

After initial shaping of lever-press responding, rats were trained under a continuous reinforcement schedule of brain stimulation using procedures identical to those described previously to evaluate opioids, cannabinoids, and nonsteroidal anti-inflammatory drugs.[35,50,51] During initial training sessions lasting 30 to 60 minutes, the white house light was illuminated, and responding produced electrical stimulation under a continuous schedule of reinforcement. Under this schedule, each lever press resulted in the delivery of a .5-second train of square-wave cathodal pulses (.1-ms pulse duration) and illumination for .5-second of the colored stimulus lights over the lever. Responses during the .5-second stimulation period did not earn additional stimulation. Initially, the frequency of stimulation was held constant