Nociceptive Responses of 3-Day-Old Rat Pups to IP Injection of Lithium Carbonate

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Various authors have suggested that neonatal responses to nociceptive stimuli are different than those observed in adults. However, our own recent observations in rat pups have indicated that neonates may be more responsive to nociceptive stimuli than previously thought. In the present study, the abdominal constrictions or writhing test of visceral nociception was adapted for use in 3-day-old rat pups in an effort to more completely assess the nociceptive responses of neonatal rat pups. Pups receiving the irritant showed robust and long-lasting behavioral differences that were attenuated by morphine sulfate. The results from our study indicate that neonatal rats pups are *not* insensitive to noxious stimulation as has been previously suggested.

Introduction

The areas of neonatal pain and pain management have recently become important and controversial topics (Anand & Hickey, 1987; Booker, 1987; Dilworth, 1988; Fletcher, 1987; Gauntlett, 1987; Harrison, 1986; Koh, 1987; Lawson, 1986; McGrath, 1988; Poland, Roberts, Gutierrez-Mazorra, & Fonhalsrud, 1987; Purcell-Jones Dorman, & Sumner, 1988; Schechter, 1989; Schoen, et al., 1989; Shearer, 1986; Silverman, 1987). Earlier reports indicate that neonates do not perceive pain as intensely as adults, have a diminished capacity to locate painful stimuli, and recover more quickly from painful insults (Bronstein, 1985; Fitzgerald & Gibson, 1984; Hatch, 1987; Mersky, 1970). Consequently, reports in the literature describe major intracavity surgical procedures being performed on unanesthetized infants (Franck, 1987; Harrison, 1986; Lawson, 1986; Shearer, 1986). In

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addition, several papers have appeared documenting the large differences in medicating practices between infants and adults undergoing similar procedures, especially when narcotic analgesic preparations are concerned (Beyer, DeGood, Ashley, & Russell, 1983; Mather & Mackie, 1983; Purcell-Jones et al., 1988; Purcell-Jones, Dorman, & Sumner, 1987; Schechter, Allen, Hanson, 1986; Yaster, 1987). For example, a recent survey of pediatric anesthetics in the UK and Ireland (Purcell-Jones et al., 1988) found that 13% of the respondents believe that neonates less than 1 week old are not able to perceive pain and an additional 7% were not sure. The overwhelming majority reported that they rarely or never prescribe analgesia during either the intra- or post-operative period regardless of surgery type, major or minor, for infants less than 1 month.

Studies utilizing animal models have also lent empirical support to the case that infants have diminished nociceptive abilities. For example, Bronstein, Mitteldorf, Sadeghi, Kirby, & Lytle (1986) have recently reported that rat pups do not show abdominal constrictions in response to challenges of hypertonic saline or acetylcholine before Day 7. Based upon these data, they have hypothesized that different nociceptive systems develop at different rates (Bronstein, et al., 1986). Furthermore, they suggest that these differences may be the result of maturational differences in sensory, motoric, or neural mechanics underlying nociception.

Neuroanatomical data indicate, however, that the nociceptive pathways are intact prenatally (for review see Anand & Carr, 1989; Anand & Hickey, 1987), and recent reports in the literature indicate that both human and animal neonates are capable of responding to noxious stimuli in a fashion similar to that observed in adults despite the relative immaturity of the central nervous system (Anand & Hickey, 1987; Fitzgerald, Millard, & McIntosh, 1989; McLaughlin, Lichtman, Fanselow, & Cramer, 1990). We have recently reported that infant rats as young as 3 days of age respond to our adaptation of the formalin test of tonic nociception in a manner remarkably similar to that observed in adult preparations, both behaviorally and in terms of their responsivity to morphine-induced antinociception (McLaughlin et al., 1990). In addition, Stickrod, Kimble, & Smotherman (1982) report that rat fetuses 20 days old react to IP injections of lithium chloride by "wriggling and contracting." Finally, although Bronstein et al. (1986) conclude that "chemically induced abdominal constrictions were *absent* in rats younger than 7 days of age" (p. 473), we have noted in unrelated studies that pups react to IP injections of lithium carbonate with abdominal constrictions and some apparent distress (McLaughlin & Cramer, 1989). These findings suggest that the neural and behavioral substrates underlying the "more complex and purposive" (Chapman, et al., 1985) nociceptive responses *are* intact in very young animals. Therefore, to more completely assess the nociceptive responses of neonatal rat pups, we have adapted the abdominal constriction or writhing to an IP chemical stimulus test of visceral nociception (Collier, Dineen, Johnson, & Schneider, 1968; Murray & Miller, 1960) for use in 3-day-old rat pups.

Two potentially important methodological differences may account for the apparent discrepancies between the Bronstein et al. (1986) report and our earlier findings (McLaughlin & Cramer, 1989). First, Bronstein and colleagues (1986) terminated their test after 60 sec if an animal did not respond. Data from adult animals, however, indicate that some substances may to be slower-acting than others (Collier et al., 1968). Second, the volume that Bronstein et al. (1986)

injected was extremely small (1 μl irritant/gm per animal). In very young rat pups a small amount of the injected volume usually leaks out because the skin is so thin. With injection volumes as small as those employed by Bronstein et al. (1986), this could significantly diminish the amount of irritant actually reaching the peritoneal cavity. In the present study, therefore, the test was extended to 30 min and the irritant diluted such that the volume could be increased by 20-fold, thus yielding injections of 20 μl irritant/gm per animal. Finally, because we have previously noted the abdominal constriction response to hypertonic solutions of lithium carbonate, that irritant was again employed in the present study.

Experiment 1

Methods

Subjects

Long-Evans-derived rat pups, 3 days of age, were used in all experiments. Females were mated in our colony with Long-Evans males (Blue Spruce, Altmont, NY). Approximately 1 week prior to parturition, the dams were housed individually in plastic tub cages (24 × 43 × 28 cm) until the conclusion of the study. The dams were checked daily in the late afternoon for pups, with the day of birth designated as Day 0. The colony was maintained on a 14 : 10 light-dark cycle at approximately 26°C with Prolab 3000 chow and tap water available ad libitum.

Procedure

Two 3-day-old rat pups were randomly selected from each litter, removed from their dam, then weighed and numbered with permanent ink. The pups were injected IP with a solution of either lithium carbonate (0.108 M, 2%-body weight) or isotonic saline. The pups were then immediately placed in Plexiglas containers (15.0 × 10.5 × 10.0 cm) and their movements videorecorded for 30 min. A heating pad set to approximately 31°C was placed under the testing chamber to keep the pups warm during testing.

The videotapes were subsequently analyzed using computer-generated time sampling (see Cramer, Thiels, & Alberts, 1990, for details). The pups' behavior was sampled and recorded at 1-sec intervals, with the observer blind to experimental treatment in both experiments. Their behaviors were categorized as: *abdominal constrictions* and *other*. Abdominal constrictions consisted of arching the back and torsional rotation of the body to one side with abdominal constrictions, behaviors typically employed in adult studies (Collier et al., 1968; Murray & Miller, 1960). The data in Experiment 1 were analyzed using a *t* test. Two pups per condition from 4 litters yielded an *N* of 8/cell.

Results

Analysis of the first 60 sec, as Bronstein et al. (1986) had previously done, revealed no significant differences in abdominal constrictions between animals injected with the lithium carbonate irritant or the isotonic saline control (mean

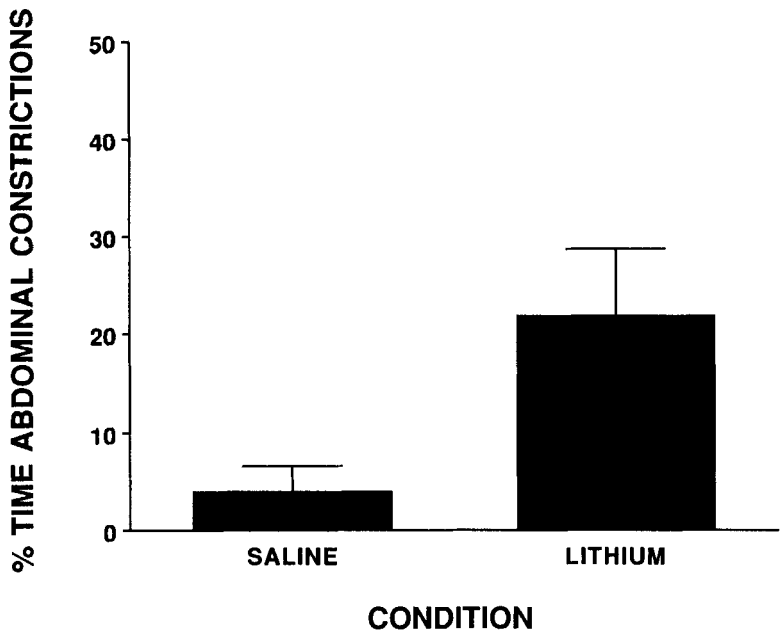

Fig. 1. Mean (±SEM) percentage of time spent engaged in abdominal constriction response during a 30-min test.

percentage time [±SEM] engaged in abdominal constrictions: lithium carbonate, 9.16% [4.39]; isotonic saline, 10.63% [4.05]; $p > 0.05$). When the entire 30 min were analyzed, however, pups receiving the irritant responded with long-lasting changes in behavior that included abdominal constrictions, arching of the back, and torsional rotation of the body to one side (Fig. 1) $t(9.8) = 2.45$, $p < 0.05$.[1] Furthermore, several pups responded to the irritant with rostral to caudal peristaltic muscular contractions, much like those previously described for older pups (Bronstein et al., 1986). This behavior is especially remarkable in light of the fact that these pups are so immature that the abdominal musculature is virtually transparent.

Experiment 2

In the second experiment, the effect of morphine on the abdominal constriction response in neonatal rats was assessed.

Procedure

Five pups were removed from each litter, weighed and numbered, following the procedure described above. The pups were injected with either morphine sulfate (2.5, 5.0, 10.0 mg/kg, IP) or isotonic saline. Twenty min after the morphine injection, all pups were injected IP with a solution of lithium carbonate (0.108 M, 2%-body weight). The behavioral scoring and testing apparatus are described in

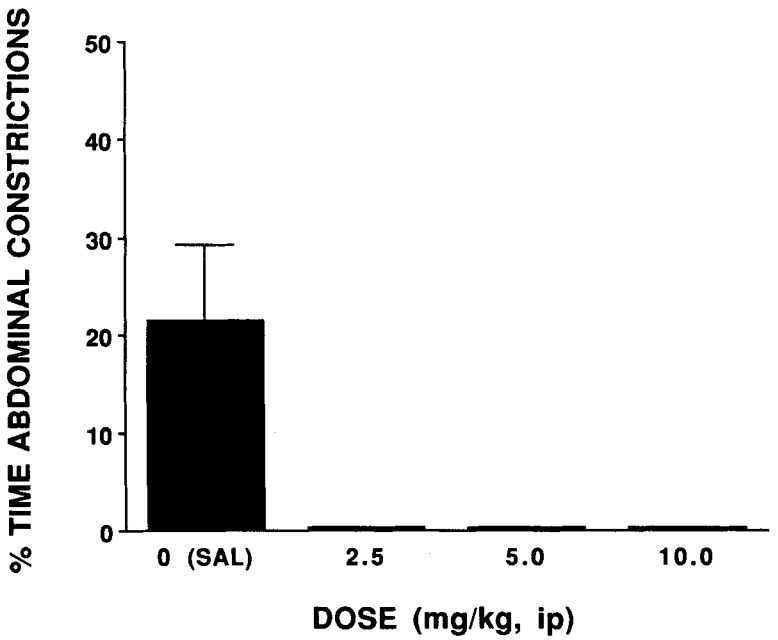

Fig. 2. The effect of morphine on mean (±SEM) percentage of time spent engaged in abdominal constriction response during a 15-min test.

Experiment 1. However, the animals in Experiment 2 were only videorecorded for 15 min to coincide with the peak morphine antinociceptive response (Johannesson & Becker, 1973). The data in Experiment 2 were analyzed using ANOVA. Twenty pups from 4 litters yielded an N of 5/cell.

Results

As can be seen in Figure 2, morphine significantly attenuated the percentage of time pups engaged in abdominal constrictions, $F(3, 16) = 7.80$, $p < 0.005$. In fact, even the lowest dose employed completely abolished the response.[2] These data indicate that even low doses of morphine suppress the behavioral response of abdominal constrictions, providing further evidence that the observed abdominal constrictions are a nociceptive response to a noxious stimulus.

General Discussion

Earlier reports suggest that the neonatal response to nociceptive stimuli is somehow different than that of adults (Bronstein, 1985; Fitzgerald & Gibson, 1984; Hatch, 1987; Mersky, 1970). Our results indicate, however, that in the abdominal constriction or writhing test of visceral nociception, neonatal rat pups exhibit robust and long-lasting behavioral responses similar to those observed in adult animals in the same test (Collier et al., 1968; Murray & Miller, 1960). The results from Experiment 2 support the assertion that the observed abdominal constrictions

are a nociceptive response to a noxious stimulus and again confirm that morphine-induced antinociception can be induced in neonatal rats.

Potentially important methodological differences may account for the discrepancies between the earlier Bronstein et al. report (1986) and the data from the present study. For example, in our study we sought to employ a dependent measure that was comparable to that previously reported in adult animals, both in behavioral topology and duration of the response (Collier et al., 1968; Murray & Miller, 1960). Bronstein et al. (1986), on the other hand, terminated their test after 60 sec if an animal did not respond, a period much shorter than studies of adult animals (Collier et al., 1968) or our data would suggest is appropriate. Second, the volume of the irritant in the Bronstein et al. study was, in our opinion, so small as to be inconsequential as an irritant. Therefore, we decreased the concentration of the salt solution in the present study so that the volume of the irritant could be increased.

In conclusion, our adaptation of the writhing test of visceral nociception demonstrates that infant rats do respond to a chemical irritant in a manner similar to that observed in adult animals indicating that they are *not* insensitive to noxious visceral stimulation as has been previously suggested (Bronstein et al., 1986).

Notes

[1] Because the variances were significantly different, $F = 7.19$, $p < 0.05$, thereby violating one of the assumptions of the t test (Hayes, 1981), our t value was calculated using the separate variance estimate which resulted in decreased degrees of freedom.

[2] Scheffe's test confirms that the 2.5, 5.0, and 10.0 mg/kg doses were significantly different from saline at the $p < 0.05$ level.

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