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Time-dependent and dose-dependent effects of fenfluramine upon pain thresholds

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Fenfluramine increases brain serotonin levels soon after injection, followed by chronic serotonin depletions 48-72 h thereafter. Because serotonin content has been implicated in both basal and analgesic nociceptive processes, the present study evaluated fenfluramine's dose-dependent and time-dependent effects upon pain thresholds in rats as measured by three tests: flinch-jump, tail flick, and liminal escape. Fenfluramine increased all three nociceptive thresholds .5 h following injection. Important test-specific differences were observed, with antinociceptive responses following effective fenfluramine doses of 2.66 mg/kg on the flinch-jump test, 13.3 mg/kg on the tail-flick test, and 26.6 mg/kg on the liminal escape test, with the last effect apparently due to overall behavioral disruption. In contrast, fenfluramine failed to alter pain thresholds 48 and 72 h after injection but significantly decreased tail-flick latencies 96 h following the lowest dose. Previous reports of morphological abnormalities following fenfluramine administration were not confirmed. While these data confirm previous reports that increases in serotonin levels produce antinociceptive effects, they do not support the contention that serotonin depletion results in hyperalgesia.

Serotonin is intricately involved in the mediation of an organism's nociceptive responsiveness (see review by Messing & Lytle, 1977). Increases in pain thresholds and decreases in brain serotonin occur in animals following (1) procedures that produce lesions in the medial forebrain bundle, septum, dorsomedial tegmentum, or nucleus accumbens, (2) systemic parachlorophenylalanine or p-chloroamphetamine injections, and (3) maintenance on tryptophan-poor diets (Gorlitz & Frey, 1972; Harvey & Lints, 1971; Lints & Harvey, 1969; Lorens, Sorenson, & Harvey, 1970; Lytle, Messing, Fisher, & Phebus, 1975; Tenen, 1967; Yunger & Harvey, 1973). Moreover, opiate analgesia is diminished by serotonin-depleting manipulations such as procedures that produce lesions in the dorsal raphe nucleus, the nucleus raphe magnus, or the dorsolateral funiculus of the spinal cord, and injections of either parachlorophenylalanine or 5,6 dihydroxytryptamine (Basbaum, Marley, O'Keefe, & Clanton, 1977; Proudfit & Anderson, 1975; Samanin, Ghezzi, Mauron, & Valzelli, 1973; Tenen, 1968; Vogt, 1974). On the other hand, serotonergic uptake inhibition and serotonergic receptor agonism increase pain thresholds (Messing, Fisher, Phebus, & Lytle, 1976; Samanin, Bernasconi, & Quattrone, 1976).

However, not all brain serotonin alterations modulate all aspects of pain thresholds. While both lesions placed in midbrain raphe nuclei and 5,7 dihydroxytryptamine injections reduce morphine analgesia, they fail to alter basal pain sensitivity (Blasig, Reinhold, & Herz, 1973; Harvey, Schlosberg, & Yunger, 1974; Hole & Lorens, 1975). Conversely, medial forebrain bundle lesions, which decrease basal pain thresholds, fail to alter opiate analgesia (Harvey et al., 1974). Furthermore, the serotonin precursors, tryptophan and 5-hydroxytryptophan, fail to increase pain thresholds (Harvey & Lints, 1971; Hole & Marsden, 1975). Finally, microiontophoresis studies revealed that morphine's analgesic effects fail to correlate with its effects upon serotonergic cell body and projection systems (Haigler, 1978).

The human appetite suppressant, fenfluramine (FEN), produces short-term increases in rodent brain serotonin levels, followed by chronic long-term depletion (Clineschmidt, Zacchei, Totaro, Pfleuger, McGuffin, & Wishousky, 1978; Harvey & McMaster, 1975) and produces irreversible morphological abnormalities in rodent median raphe serotonergic cells (Harvey & McMaster,

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1975, 1977; Harvey, McMaster, & Fuller, 1977). Therefore, if nociceptive sensitivity is altered by changes in serotonin levels, then FEN administration should produce short-term analgesia followed by hyperalgesia 48-72 h thereafter. Thus, Experiment 1 determined FEN effects upon rodent pain thresholds, including reactivity to electric shock (the flinch-jump test), radiant heat (the tail-flick, TF, test), and operant psychophysical thresholds (the liminal escape, LE, test). The last procedure has been shown to reflect both an organism's evaluation of the relative aversiveness of a given stimulus and its motivation to respond, or not, to terminate the stimulus (Bodnar, Kelly, Brutus, Mansour, & Glusman, 1978). In addition, the intertrial interval behavior generated by this procedure reliably indicates alterations in the organism's ability to respond and thereby serves to quantify the relative influence of nonspecific factors in the analgesic response. Experiment 2 examined whether FEN treatment produces morphological abnormalities in brain tissue.

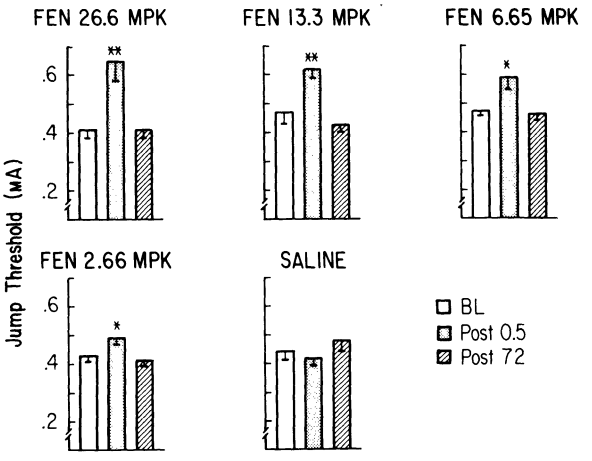

Figure 1. Mean alterations in jump thresholds (+SEM) .5 and 72 h following acute exposure to either fenfluramine (FEN) at doses of 26.6, 13.3, 6.65, and 2.66 mg/kg or saline. There were six animals in each group.

EXPERIMENT 1

Method

Seventy-three male albino Sprague-Dawley rats were housed singly and were maintained on an ad-lib diet of rat chow and water. Animals were placed on a 12-h light/12-h dark cycle and were tested 1-7 h into the light cycle. Flinch-jump thresholds were determined in a modification of Evans' (1961) procedure and are described in detail elsewhere (Bodnar et al., 1978). TF latencies (D'Amour & Smith, 1941) were determined using a radiant heat source (IITC analgesia meter) mounted 8 cm above the tail of a restrained animal. The thermal stimulus was applied to the dorsum of the tail 4 cm proximal to the tip, and its intensity was adjusted to produce a baseline TF latency of 3-4 sec. Three trials, separated at 30-sec intervals, were run daily.

The contingency of the LE test, described in detail elsewhere (Bodnar et al., 1978), was such that footshock was delivered for 10 sec unless the rat pressed a lever three times to initiate a 20-sec intertrial interval. One hundred trials were distributed evenly over a session and across intensities (.2, .4, .6, .8, 1.0 mA) that were tested in blocks of four trials in counterbalanced fashion. The probability and latency to escape were recorded separately at each intensity for the last 80 trials of the session. Intertrial behavior was determined by the amount of time (in seconds) that contact was made with the lever during the intertrial interval.

Four days of baseline determinations were completed for each test. Flinch-jump thresholds were then determined .5 h and 72 h following respective intraperitoneal injections of FEN at doses of 0, 2.66, 6.65, 13.3, and 26.6 mg/kg (1 ml normal saline/kg body weight) in five matched groups of six animals each. Twenty other animals, divided into five matched groups of four rats each, received FEN at the five stated doses, with TF latencies determined .5, 24, 48, 72, and 96 h following the injections. To avoid tissue damage in testing TF latencies, the trials were automatically terminated if a withdrawal response to the heat stimulus did not occur within 6 sec. Finally, 23 rats matched for LE thresholds received either FEN at a dose of 26.6 mg/kg (n = 14) or a vehicle (saline) (n = 9), with thresholds determined .5, 24, 48, and 72 h later.

Results

Flinch-jump test. Figure 1 shows that while jump thresholds were increased in a dose-dependent manner .5 h following FEN, they failed to decrease below control values 72 h thereafter. Significant differences were observed over time [$F(2,50) = 20.69$, $p < .001$] for the interaction between groups and time [$F(8,50) = 4.53$, $p < .01$], but not among groups [$F(4,25) = 1.12$]. FEN significantly increased jump thresholds .5 h following administration at all doses tested: 26.6 [$t(10) = 3.09$, $p < .05$], 13.3 ($t = 6.19$, $p < .01$), 6.65 ($t = 2.85$, $p < .05$), 2.66 mg/kg ($t = 3.20$, $p < .05$). By contrast, neither vehicle treatment .5 h prior to testing ($t = .72$) nor any FEN dose 72 h following treatment significantly altered jump thresholds.

TF test. Figure 2 shows that while latencies were increased in a dose-dependent manner .5 h following the injection, they failed to decrease below control levels 72 h thereafter, except for the lowest FEN dose. Significant differences were observed over time [$F(5,75) = 15.56$, $p < .01$] for the interaction between groups and time [$F(20,75) = 2.48$, $p < .01$], but not among groups [$F(4,15) = .97$]. FEN significantly elevated latencies .5 h after injection at doses of 26.6 [$t(6) = 5.38$, $p < .01$] and 13.3 mg/kg ($t = 8.88$, $p < .01$), but it failed to alter latencies following the 6.65- ($t = .88$), 2.66- ($t = 1.15$), and 0-mg/kg ($t = .18$) doses. Moreover, FEN failed to alter latencies 24, 48, 72, and 96 h following the injection, except for a significant decrease induced by the lowest dose after 96 h ($t = 5.79$, $p < .01$).

LE test. Figure 3 illustrates the time-dependent alterations in LE thresholds following FEN injection. Significant differences in time spent in shock were observed between FEN- and saline-treated groups [$F(1,21) = 4.33$, $p < .05$] across time [$F(4,84) = 32.13$, $p < .001$] and across intensities [$F(4,84) = 199.51$, $p < .001$]. Significant interactions were observed among all combinations of variables. Alterations in escape probability yielded an identical pattern. FEN-treated rats exhibited significant

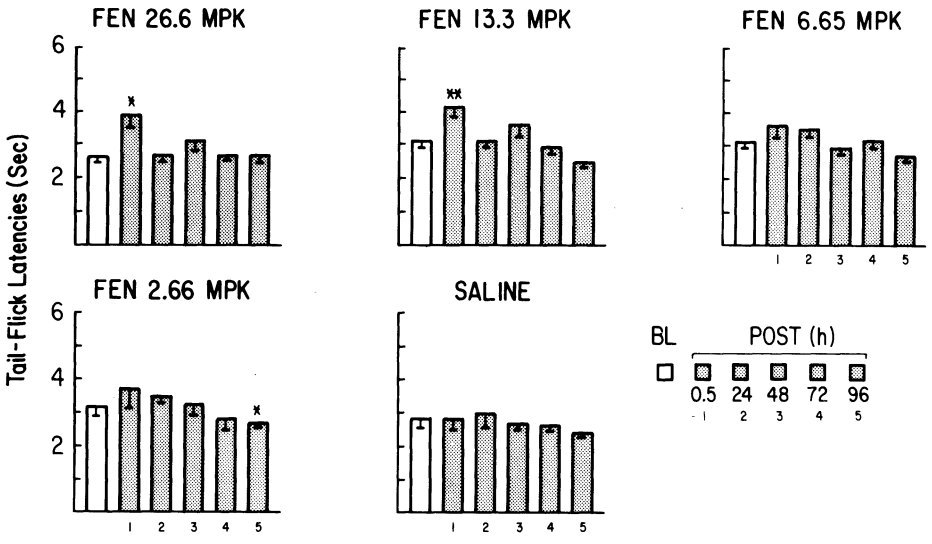

Figure 2. Mean alterations in tail-flick latencies (+SEM) .5, 24, 48, 72, and 96 h following acute exposure to either fenfluramine (FEN) at doses of 26.6, 13.3, 6.65, and 2.66 mg/kg or saline. There were four animals in each group.

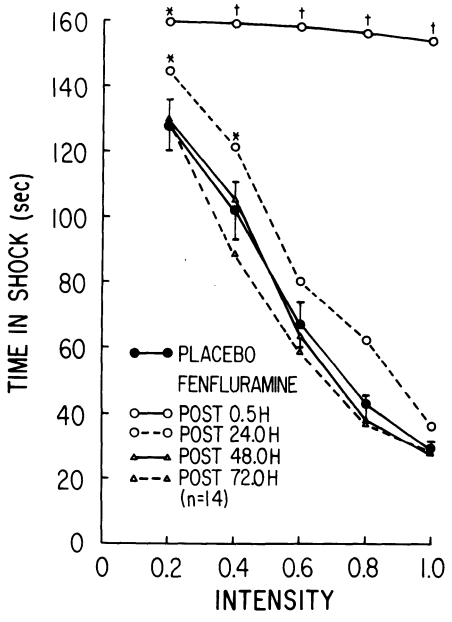

Figure 3. Time course of mean alterations in liminal escape thresholds across shock intensities for 14 rats treated with a 26.6-mg/kg dose of fenfluramine. The SEM of the placebo baseline for each intensity is displayed.

increases across the entire LE function .5 h after injection, with the majority of animals failing to emit any appropriate escape or intertrial behaviors. On the other hand, saline-treated rats exhibited normal escape functions. Both LE thresholds and intertrial behaviors returned to within normal limits thereafter, except for significant increases at low shock intensities 24 h after injection.

EXPERIMENT 2

Method

Randomly selected rats that received either the 0- (n = 3) or the 26.6-mg/kg FEN dose (n = 6) were anesthetized with sodium pentobarbital and were perfused trancardially with .9% normal saline and 10% formalin. The brains were quickly removed, blocked, fixed for 7 days, embedded in paraffin, and sliced in 5-micron coronal serial sections. Five of every 15 sections from the diencephalon to the caudal medulla were mounted and stained with cresyl violet. Light-microscopic examination of the tissue for morphological abnormalities was done by an experienced anatomist who had no knowledge of the behavioral results or treatment.

Results

No consistent degeneration of cells was observed in the medullary, pontine, and midbrain raphe nuclei, the locus coeruleus, the oculomotor (IIIrd) and trigeminal (Vth) cranial nerves, the substantia nigra, or the periaqueductal gray and the pontine reticular formation in FEN-treated rats. Except for occasional (one to two cells per section over three to four sections) clumping of glia around several neurons in the median raphe nucleus of two FEN-treated rats, no abnormalities were noted. These data are in accord with other recent reports that failed to find systematic abnormalities (Funderburk, 1977; Gibbons, Glusman, Barr, Bridger, & Leibowitz, 1978).

DISCUSSION

The hypotheses that pain thresholds would increase soon after FEN injection and subsequently decrease were partially confirmed. FEN increased nociceptive thresholds on all three pain tests in a dose-dependent manner .5 h following injection, a time when FEN increases brain serotonin levels (Clineschmidt et al., 1978). Differences in effective analgesic FEN doses were observed

for the flinch-jump thresholds (2.66 mg/kg), TF latencies (13.3 mg/kg), and LE thresholds (26.6 mg/kg) .5 h following injection, with the last effect attributable to disruption of overt behaviors as confirmed by elimination of ongoing intertrial behaviors that reliably indicate changes in motor performance (Dinsmoor, Matsuoka, & Winograd, 1958; Davis & Burton, 1974) but not analgesic performance (Bodnar et al., 1978). FEN failed to alter intertrial behaviors following the lower doses, indicating that the observed antinociceptive responses were not due to nonspecific factors at these levels.

The present study also demonstrated that FEN failed to lower basal flinch-jump, TF, and LE thresholds 48-72 h after injection (except for a small but significant decrease in TF latencies 96 h following the lowest FEN dose), despite its effects of long-term serotonin depletions (Clineschmidt et al., 1978). These data agree with the failure of serotonin content depletion to decrease basal pain thresholds observed in some (Blasig et al., 1973; Harvey et al., 1974; Hole & Lorens, 1975), but not all (Tenen, 1967; see review by Messing & Lytle, 1977), studies. One explanation for these discrepancies includes the possibility that serotonin must be reduced to some minimal critical level at specific loci to induce hyperalgesia. Alternatively, procedures (such as those that produce diencephalic and telencephalic lesions) that concomitantly lowered pain thresholds and brain serotonin (Harvey & Lints, 1971; Lints & Harvey, 1969; Lorens et al., 1970; Yunger & Harvey, 1973) also interrupted other neurotransmitter and neuropeptide pathways. It is concluded that FEN increases pain thresholds in a test-specific fashion, but whether this analgesia is due to release of serotonin is open to further study.

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