Protracted analgesia in young and adult rats maternally exposed to methadone[1]

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Summary. The analgesic response to the hot-plate test was studied in 21-, 45-, 60-, 120- and 300-day-old rats maternally exposed to methadone (5 mg/kg). An elevation in nociceptive threshold, in the absence of further exposure to methadone, was observed in young and adult rats perinatally subjected to methadone.

Methadone is a synthetic analgesic that is widely used for social rehabilitation and adjustment of chronic heroin users[2], many of whom are women of childbearing age[3]. Clinical and laboratory studies[2-9] have shown that methadone crosses the placenta and enters the fetal circulation; moreover, this drug has been detected in the milk of lactating humans on methadone maintenance[10]. Although neonates delivered by mothers receiving methadone are known to undergo the withdrawal syndrome[3], and these children often have a retardation in body growth and exhibit behavioral abnormalities[5,11-13], very little is known about the short- and long-term consequences of perinatal drug exposure on later life. Evidence gathered in a series of morphological, biochemical, and behavioral investigations[6,14-20] suggests that methadone exposure during gestation and/or lactation: a) affects neurobiological maturation, often producing permanent deficits in cell number, b) is associated with subnormal motor activity at weaning, but with hyperactivity in juvenile and young rats, and c) impairs adult learning ability. The present study demonstrates that exposure to methadone during perinatal life is related to a prolonged analgesia that lasts into adulthood.

Materials and methods. Female (180-200 g) Sprague-Dawley rats were housed under standard conditions[15-17] and treated daily (08.00 h) with an i.p. injection of either 5 mg/kg dl-methadone hydrochloride (Dolophine, Eli Lilly Co., Indianapolis, IN) or an equivalent volume of physiologic saline. 5 days after the beginning of treatment, females were mated. All injections were continued throughout mating, gestation, and lactation, and terminated at weaning (day 21). Within 4 h after birth, 4 groups of animals were established as described elsewhere[15,17]. One group of animals was exposed to methadone only during gestation, another group received methadone only during lactation, and a 3rd group was continuously subjected to methadone throughout gestation and lactation; litters from saline-injected mothers served as controls. 6 males and 6 females per group were tested for analgesia at 21, 45, and 60 days, while 6-9 females and 4 females per group were examined at 120 and 300 days of age, respectively. The hot-plate technique of Woolfe and McDonald[21] was used in this study. Animals were placed on a heated surface maintained at 66 °C and the latency of their reflex response to heat was recorded to the nearest 0.1 sec with a Camero stopwatch (Switzerland). Animal responses employed as endpoints included: licking of the paws, withdrawal of one of the hindlimbs from the plate, or jumping off from the top of the plate. Any subject not responding within 15 sec was removed from the hot-plate.

Results. At all ages examined, rats perinatally subjected to methadone were slower to respond to the hot-plate than controls (table) and, in some cases, these animals remained on the hot-plate for nearly twice as long as controls (e.g., gestation and gestation-lactation groups at 45 days). Rats receiving methadone throughout gestation and lactation were markedly affected at every examination period and often had the longest latency times; for example their reaction times were 3-fold greater than controls at day 60. In comparison to controls, animals in the gestation and lactation groups each recorded significantly slower reactions to the hot-plate than controls at 3 ages, while all methadone groups had longer latencies of response on days 45 and 120. At 21, 45, and 60 days, no sex differences were observed in control or methadone-treated groups.

Discussion. The results of this study reveal that rats, maternally subjected to methadone, had an elevation in nociceptive threshold on the hot-plate test that was present long after cessation of drug exposure and continued into adult life. This analgesia was observed in the absence of any additional challenges with methadone. Moreover a prolonged period of analgesia was recorded in animals treated with methadone under a variety of treatment schedules, but was most evident in rats given a cumulative exposure (i.e., gestation-lactation groups). In fact, animals in this group were still significantly analgesic 279 days after drug exposure.

The mechanism(s) whereby methadone exposure in early life elicits a protracted analgesia that persists long after

Analgesic response to the hot-plate test of young and adult rats maternally subjected to methadone

Treatment schedule	AGE (days)				
	21	45	60	120	300
Control	0.83±0.05 (0.6–1.2)	0.55±0.12 (0.3–0.7)	1.23±0.12 (0.7–2.0)	0.84±0.04 (0.6–1.0)	1.23±0.21 (0.8–1.8)
Gestation	0.97±0.06* (0.8–1.2)	1.02±0.11** (0.7–1.4)	1.35±0.17 (0.7–2.6)	1.11±0.06* (0.8–1.3)	1.65±0.25 (1.0–2.2)
Lactation	0.88±0.04 (0.8–1.2)	0.89±0.05** (0.8–1.2)	1.90±0.28* (1.0–4.0)	0.96±0.08* (0.7–1.3)	1.65±0.22 (1.1–2.2)
Gestation/lactation	1.08±0.06** (0.8–1.6)	1.07±0.07** (0.8–1.3)	3.92±0.40** (2.3–6.3)	0.92±0.40* (0.9–1.0)	2.50±0.10* (2.3–2.6)

Values represent mean latency scores (sec± SE) for animal response to the hot-plate test; range of values (sec) in parentheses. N = 12 animals (equal number of males and females) per group at 21, 45, and 60 days of age, N = 6–9 females per group at 120 days of age, and N = 4 females per group at 300 days of age. Significantly different from controls at $p < 0.05$ and $p < 0.01$ using the Dunnett's procedure[22].

termination of drug treatment is unclear. Since methadone has been shown to cause a variety of morphological and biochemical alterations of the brain[14-17], it might be conjectured that CNS and/or PNS structures associated with nociceptive mechanisms are damaged during the developmental period. Alternatively, since methadone has been reported to accumulate in the brains of preweaning animals[8], and a single s.c. injection of methadone has been found to persist in the adult brain for at least 3 weeks[7], perhaps methadone remaining in the brain from chronic perinatal drug treatment is effecting the continued state of analgesia.

Although it is difficult to generalize between our laboratory data and clinical situations, it seems appropriate to raise the question of whether perinatal exposure to methadone (either transplacentally or during breastfeeding) could interfere with pain perception during childhood and later stages of life. In fact, other sensory modalities may also be impaired in these drug-exposed individuals. In view of the social and emotional implications of such findings, as well as the importance of these results to such areas as the clinical management of pain, the relationship between maternal methadone consumption and the physiological integrity of their progeny needs to be evaluated.

1 This research was supported by grant DA 01618.
2 V.P. Dole and M.E. Nyswander, J. Am. med. Ass. *193*, 646 (1965).
3 G. Blinick, R.C. Wallach, E. Jerez and B. Ackerman, Am. J. Obstet. Gynec. *125*, 135 (1976).
4 G. Blinick, C.E. Inturrisi, E. Jerez and R.C. Wallach, Am. J. Obstet. Gynec. *121*, 617 (1975).
5 A. Lodge, M.M. Marcus and C.M. Ramer, Int. J. Addict. *2*, 235 (1975).
6 P.J. McLaughlin, I.S. Zagon and W.J. White, Biologia Neonat. *34*, 48 (1978).
7 A.L. Misra and S.J. Mule, Nature *238*, 155 (1972).
8 M.A. Peters, J. Pharmac. exp. Ther. *192*, 513 (1975).
9 M.A. Peters, M. Turnbow and D.J. Buchenauer, J. Pharmac. exp. Ther. *181*, 273 (1972).
10 G. Blinick, E. Jerez and R.C. Wallach, J. Am. med. Ass. *224*, 477 (1973).
11 C.M. Ramer and A. Lodge, Int. J. Addict. *2*, 227 (1975).
12 R.Y. Ting, A. Keller and L.P. Finnegan, Proc. 2nd nat. Drug Abuse Conf., New Orleans 1975.
13 G. Wilson, Addict. Dis. *2*, 333 (1975).
14 I.S. Zagon and P.J. McLaughlin, Biologia Neonat. *31*, 271 (1977).
15 I.S. Zagon and P.J. McLaughlin, Exp. Neurol. *56*, 538 (1977).
16 I.S. Zagon and P.J. McLaughlin, Experientia *33*, 1486 (1977).
17 I.S. Zagon and P.J. McLaughlin, J. Neurochem. *31*, 49 (1978).
18 I.S. Zagon and P.J. McLaughlin, Pharmac. Biochem. Behav. *9*, 665 (1978).
19 I.S. Zagon, P.J. McLaughlin and C.I. Thompson, Pharmac. Biochem. Behav. *10*, 743 (1979).
20 I.S. Zagon, P.J. McLaughlin and C.I. Thompson, Pharmac. Biochem. Behav. *10*, 889 (1979).
21 G. Woolfe and A.D. McDonald, J. Pharmac. exp. Ther. *80*, 300 (1944).
22 B.J. Winer, Statistical Principles in Experimental Design. McGraw-Hill, New York 1971.

Potentiation of acoustic-trauma-induced audiogenic seizure susceptibility by salicylates in mice[1]

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Summary. Combined exposure to noise and salicylates was found to produce greater acoustic trauma induced audiogenic seizure risk than exposure to the noise alone. The result suggests that salicylates could make the mouse cochlea more vulnerable to the traumatic action of noise.

There are 2 major types of ototraumatic agents in our environment: various types of noise and ototoxic drugs. The ototoxic drugs may be classified into 2 categories. Irreversible ototoxic drugs such as kanamycin, neomycin or streptomycin, can cause irreversible damage to the inner ear structures resulting in a permanent loss of hearing, whereas reversible drugs such as quinine, salicylates or etacrynic acid usually produce a transient hearing loss, and recovery generally occurs after cessation of treatments.

An important practical, as well as theoretical, issue is whether simultaneous exposure to these 2 ototraumatic agents, i.e. intense noise and drugs, can result in the mutual potentiation of their ototraumatic effects. Available evidence appears to suggest that it can, when irreversible ototoxic drugs are used[2]. However, it is not clear if this interactive effect can be obtained when reversible ototoxic drugs are used. It has been concluded in a recent review paper that noise and salicylates do not interact and therefore salicylates should not pose additional hazards to organisms exposed to intense noise[2]. This report presents data suggesting that salicylates could in fact potentiate ototraumatic effects of noise in mice.

A high incidence of audiogenic seizures may be induced in genetically seizure resistant (BALB/c) mice by exposure to an intense noise a few days prior to testing for seizures[3]. This phenomenon has been termed priming for audiogenic seizure susceptibility. Available evidence suggests that the principle effect of the priming exposure is to cause stimulation damage to the cochlea and that this damage is the primary underlying condition for the development of susceptibility to seizures[4].

The major evidence supporting this contention includes the following: 1. acoustically primed mice tend to show a reduction of cochlea microphonic responses[4]; 2. extensive damage to outer hair cells can be produced by effective priming exposure[5]; 3. seizure susceptibility can be induced by ototoxic drugs such as kanamycin[5] and 6-aminonicotinamide[6]; 4. the effectiveness of a priming stimulus is an increasing function of its intensity[7], exposure duration[8], and acoustic energy[9], all of which are well known parameters of stimulation damage to the cochlea[10].

In view of these findings, it is reasonable to assume that priming for audiogenic seizures is a valid indirect method for evaluations of the ototraumatic effects of noise. The present study was designed to investigate whether exposure to an intense noise while under salicylate intoxication would result in a greater priming effect than exposure to the noise alone.