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Age-Related Responsiveness of the Rat to Drugs Affecting the Central Nervous System[1] (38395)

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With few exceptions, the preclinical evaluation of drugs is undertaken in healthy, young adult animals. The available literature on the interaction between the age of animals and drug effects are largely concerned with the age span from neonate to young adult, *i.e.*, developmental studies. Kato and Takanaka (1) have demonstrated depressed *in vitro* drug metabolism of several drugs including hexobarbital and meprobamate in 250–600 day old rats as compared to 100 day old rats. These investigators (2) also found an increased duration of action and a decreased rate of decline of plasma pentobarbital levels with increasing age. Ziem *et al.* (3) have reported increased central nervous system sensitivity to amphetamine in 12–14 mo old rats when compared with 3 mo old animals.

The work presented here was performed to demonstrate age-related alterations in the responsiveness of mature rats to centrally acting drugs and to emphasize the importance of regarding the age of the animal as a significant factor in assessing drug response. Responses to sodium hexobarbital, chlorpromazine HCl, morphine SO_4 and *d*-amphetamine SO_4 were evaluated in 2.5–3 mo old and 9–10 mo old male rats.

Methods. Male, albino Cox/Sprague–Dawley rats were obtained from Laboratory Supply Co., Indianapolis and housed in community cages for 1–2 weeks prior to use. Wayne Lab Bloks and tap water were supplied *ad libitum*. Room lights were cycled from 6 AM to 8 PM daily and temperature was kept constant at 25 ± 1.0°.

The electroencephalogram was recorded from three cortical electrodes (SS fillister head machine screws 0-80 × 1/8 in.) implanted 2 mm to either side of the saggital suture and 2 mm posterior to the bregma. A third screw was placed 1 cm anterior to the bregma and 1 mm to the right of the saggital suture. The latter served as the ground connector for the preamplifier. Cranioplastic cement was employed to provide support and insulation for the contacts. Twenty-four hours later, an indwelling jugular cannula (PE-20) was put in place and kept patent with heparinized 0.9% NaCl solution. All surgical procedures performed employed ether as the anesthetic. EEG recordings were made on a Physiograph, Model DMP-4a. The suppression of EEG activity lasting at least 1 sec — "silent second" (4) during an iv infusion of hexobarbital Na (15 mg/kg/min/ was taken as a measure of central nervous system sensitivity. When this endpoint was reached, the animals were euthanized and brain and plasma were collected for the spectrophotometric determination of hexobarbital levels (5).

For the chlorpromazine (CPZ)[2] hypothermia study, the animals were placed in individual cages in a constant temperature room maintained at 18° and 40% relative humidity 24 hr prior to CPZ administration. Core temperatures were monitored hourly with a thermister probe inserted into the rectum.

Morphine-induced analgesia was assessed by measuring the amount of electrical current delivered as foot shock required to elicit a vocalization response (vocalization threshold). Intraperitoneal doses of 5, 7, 10 and 14 mg/kg were employed. Current was delivered to the animal at 1.5 sec intervals as 0.5 sec duration trains of 50 μsec square pulses of alternating sign using a Grass brief pulse stimulator. The intensity of the

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[2] Chlorpromazine HCl was graciously supplied by Smith Kline and French Labs.

current was increased for each train in 0.1 mA steps from 0.4 to 1.9 mA. Intertrial intervals were 15 sec.

D-amphetamine sulfate was included as a typical central stimulant. Photoelectric activity cages (Woodard) were used to measure the effect on locomotor activity. The animals were allowed 100 min to become accustomed to the cages prior to the ip administration of amphetamine 0.5, 1.0 and 2.0 mg/kg. Activity occurring between 10 and 100 min following drug administration was recorded.

Results. The dose of hexobarbital required to produce the "silent second" was significantly lower in the older animals (59.1 ± 5.0 mg/kg vs 74.1 ± 2.0 mg/kg, $P < 0.02$). Since the endpoint appears in less than 5 min, differences in the rates of drug metabolism in the two age groups would have only minimal influence. Although the older animals received a lower dose of drug the brain levels were not significantly different in the two age groups (19.1 ± 2.6 μg/g vs 17.2 ± 2.1 μg/g) and the plasma levels were slightly elevated in the older animals (127.0 ± 8.5 μg/ml vs 104.0 ± 7.7 μg/ml, $0.10 > P > 0.05$). These findings suggest that the distribution of the drug is altered in the older animals.

The time course of hypothermia induced by 10 mg/kg of CPZ, ip, was determined in 3 and 10 mo old animals (Fig. 1). Body temperatures immediately prior to drug administration were not different. The hypothermia was significantly greater in older animals after 2, 3 and 4 hr. In a subsequent experiment, brain CPZ levels were determined gas chromatographically (6) 2.5 hr after drug administration and body temperatures were also recorded. The hypothermia was again greater in the older animals (3.81 ± 0.45° vs 2.39 × 0.29° $P < 0.05$), but no difference was seen in the brain levels (0.56 ± 0.05 μg/g vs 0.66 ± 0.08 μg/g, $P > 0.25$). These data suggest that the sensitivity of the central nervous system to CPZ may increase with age, although peripheral actions of the drug may be contributing to the effect.

As shown in Fig. 2, the older animals were more sensitive to morphine. The slopes of the regression lines are 11.2 for the 10 mo old animals and 16.0 for the 3 mo old animals and are significantly different ($P < 0.001$). There was no difference between the two age groups following saline injection.

Sensitivity to amphetamine was also greater in the older animals (Fig. 3). The slopes of the regression lines for the 9 mo old animals and for the 2.5 mo old animals are significantly different ($P < 0.01$). Both age groups showed similar levels of activity following saline injection.

Discussion. Age is usually regarded as an experimental variable only when some of the animals are immature. The work presented here clearly demonstrates that age-related changes in responses of rats to drugs continue to occur after the animals have matured. The control responses

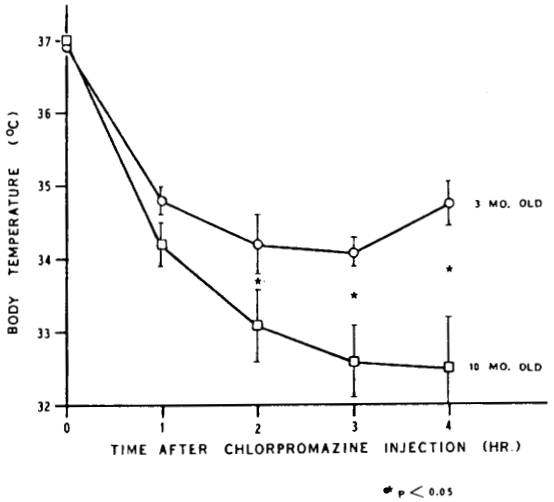

FIG. 1. Time course of chlorpromazine hypothermia in 3 and 10 mo old rats. Colonic temperature was monitored following the ip administration of 10 mg/kg chlorpromazine HCl. Each point represents the mean of six animals ± SE.

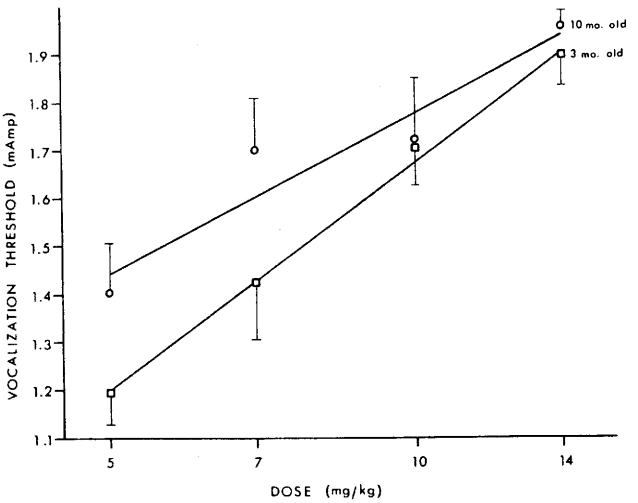

FIG. 2. Effect of morphine on the vocalization threshold in 3 and 10 mo old rats. The amount of electrical current delivered as foot shock required to elicit vocalization (vocalization threshold) was determined 40 min after the ip administration of morphine sulfate. Each point represents the mean of five animals ± SE.

which were measured, *viz.*, body temperature, spontaneous motor activity and pain thresholds were not different in the two age groups studied. When the animals were challenged with the drugs (chlorpromazine, amphetamine and morphine), however, a significantly higher responsiveness to the drugs occurred.

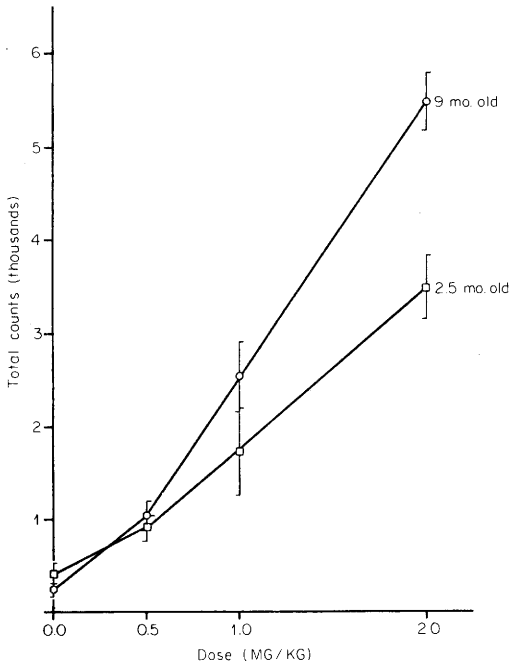

FIG. 3. Amphetamine-stimulated motor activity in 2.5 and 9 mo old rats. Activity was measured in photoelectric activity cages for a 90 min period commencing 10 minutes after the ip administration of *d*-amphetamine sulfate. Each point represents the mean of 6 animals ± SE.

The older animals used in these studies were in no sense "old," yet differences in responsiveness between the age groups of approximately 3 and 9 mo old rats were evident. Several mechanisms are probably involved in this phenomenon including alterations in drug distribution, metabolism, elimination and target organ sensitivity. The latter is accented in this study by a smaller dose of hexobarbital required to reach the EEG "silent second." In pharmacologic studies, the age of the animals employed should be considered as one of the experimental variables to be controlled and reported.

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