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Chloral hydrate anesthesia antagonizes the neurotoxicity of 3,4-methylenedioxymethamphetamine

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We report that maintaining rats under chloral hydrate anesthesia for the first 3 h following the administration of 3,4-methylenedioxymethamphetamine (MDMA) blocks the decrease in forebrain concentrations of 5-hydroxytryptamine (5-HT) measured 1 week later. In contrast, the acute effect of MDMA (3 h) on forebrain 5-HT was not altered by the anesthetic. This protective effect of chloral hydrate was not due to an anesthetic-induced hypothermia but may be related to the hypothesized role of dopamine in the neurotoxic effects of MDMA.

Amphetamine; MDMA (3,4-methylenedioxymethamphetamine); Chloral hydrate

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA) is a popular recreational drug known to abusers as ecstasy. When administered at high doses to laboratory animals, MDMA is a selective serotonergic neurotoxin. The unique subjective effects of MDMA have brought it to the attention of psychopharmacologists while its neurotoxicity has made it the focus of numerous neuropharmacological studies. Recent reports have implicated dopamine release as a necessary event in the development of MDMA-induced neurotoxicity (Stone et al., 1988; Schmidt et al., 1990b).

The early neurochemical effects of MDMA are responsible not only for the behavioral effects of the drug but may also initiate the sequence of events leading to its neurotoxicity. One of the earliest effects of MDMA, like other amphetamines, is an increase in the release of monoaminergic neurotransmitters. As predicted from studies using tissue slices (Johnson et al., 1986; Schmidt et al., 1987), Yamamoto and Spanos (1988) demonstrated that MDMA produced a dose-dependent increase in striatal dopamine release in the awake, behaving rat. In contrast to these results, a more recent study by Gazzara et al. (1989) found a decrease in extracellular dopamine in the striatum following the administration of similar doses of MDMA. The primary difference between these two studies was the use of chloral hydrate-anesthetized rats in the latter work. Based upon these observations we speculated that if chloral hydrate interfered with MDMA-induced dopamine release and if dopamine release was involved in the neurotoxic effects of MDMA, then chloral hydrate anesthesia may block the development of MDMA-induced neurotoxicity.

2. Materials and methods

Male Sprague-Dawley rats (approximately 250 g) were randomly placed into two groups receiving either saline of chloral hydrate (400 mg/kg i.p.). MDMA (20 mg/kg s.c. as the free base) was

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administered to half of the animals in each group 30 min later. Supplemental doses of 30-100 mg/kg chloral hydrate were used to maintain a state of unconsciousness and immobility for a duration of 3 h after which animals were immediately killed or returned to the animal facility for 1 week. Following decapitation, brain regions were removed on ice and stored at −70°C. Regional brain concentrations of the monoamines and their metabolites were determined by HPLC with electrochemical detection (Schmidt et al., 1987). In one experiment, Deltaphase isothermal pads (Braintree Scientific, Braintree, MA) were used to maintain body temperature of the rats for the duration of the anesthesia. Rectal temperatures were monitored by means of a RET 2 thermocouple probe with a BAT 10 Thermometer (Sensortek, Clifton NJ). Results from all long-term experiments were combined and analyzed by one-way analysis of variance and the Student's-Newman-Keuls test using a significance level of $P < 0.05$. Results from temperature measurements were compared using an analysis of variance for repeated measurements with a subsequent least-squares means test for individual differences.

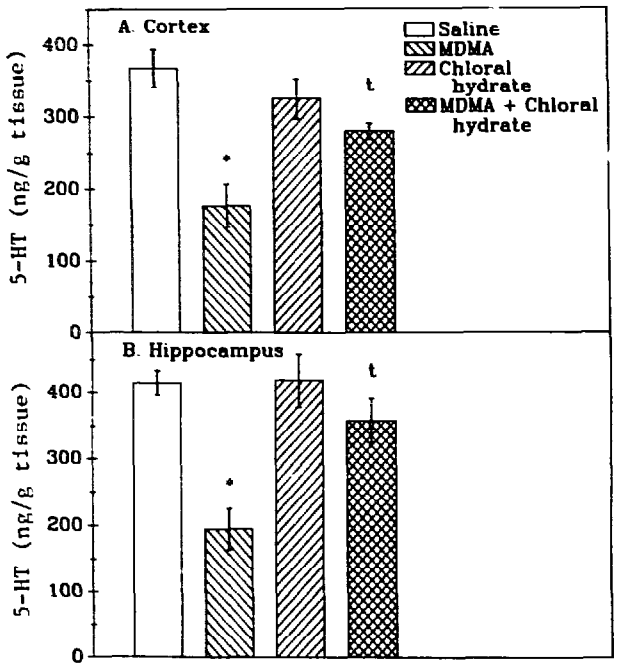

Fig. 1. Effect of 3 h of chloral hydrate anesthesia on the depression of cortical (A) and hippocampal (B) 5-HT concentrations observed 1 week after the administration of 20 mg/kg MDMA. Each value is the mean ± S.E.M. for five or more animals. *$P < 0.05$ vs. saline; †$P < 0.05$ vs. MDMA.

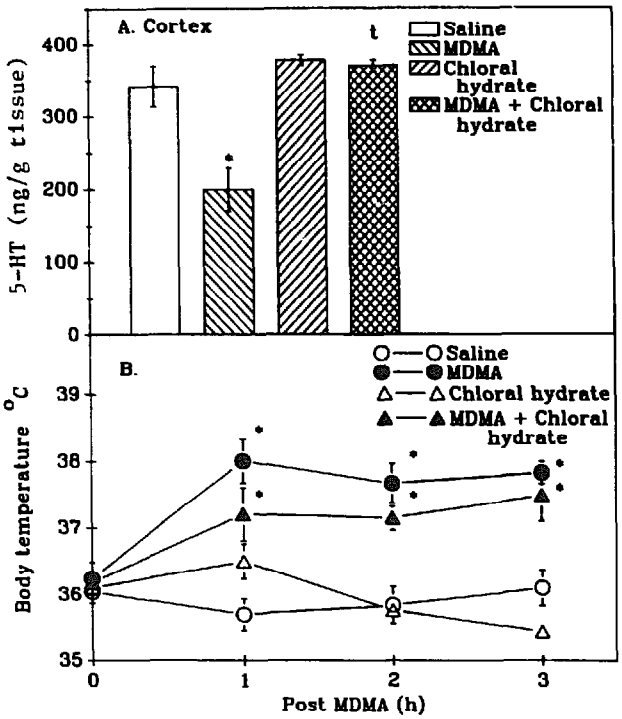

Fig. 2. MDMA-induced hyperthermia in control and chloral hydrate-anesthetized rats maintained on isothermal pads is shown in (B). (A) Shows the resultant effect of MDMA on cortical 5-HT concentrations 1 week later. The dose of MDMA was 20 mg/kg. Each point is the mean ± S.E.M. for three or more animals. *$P < 0.05$ vs. saline; †$P < 0.05$ vs. MDMA.

3. Results

Figure 1 provides results from experiments in which MDMA (20 mg/kg s.c.) was administered to control or chloral hydrate-anesthetized rats 1 week prior to killing. The neurotoxic effect of MDMA on serotonergic neurons is illustrated by the average 50% decline in both cortical (A) and hippocampal (B) 5-hydroxytryptamine (5-HT) concentrations measured in the control group. Maintaining animals under chloral hydrate anesthesia for the first 3 h following drug administration significantly reduced the long-term depletion of forebrain 5-HT by MDMA. Identical effects were observed in the striatum (not shown).

TABLE 1

Changes in striatal monoamines 3 h after MDMA (10 mg/kg) administration to saline- or chloral hydrate-anesthetized rats.

	Dopamine (ng/g)	DOPAC (ng/g)	HVA (ng/g)	5-HT (ng/g)	5-HIAA (ng/g)
Saline	9592±497 (100± 5)	1103±79 (100± 7)	721±53 (100± 7)	333±11 (100± 3)	398±45 (100±11)
MDMA	11845±593 [a] (123± 6)	602±69 [a] (55± 6)	730±94 (101±13)	146± 9 [a] (44± 3)	292±13 (73± 3)
Chloral hydrate	9686±724 (101± 8)	970±38 (88± 3)	860±47 (119± 7)	370±23 (111± 7)	520±30 (131± 8)
MDMA + chloral hydrate	13254±663 [a] (138± 7)	519±51 [a] (47± 5)	876±32 [a] (122± 4)	192±14 [a] (58± 4)	391±18 (98± 5)

[a] $P < 0.05$ vs. saline.

We have recently demonstrated that hypothermia can interfere with the long-term serotonergic deficits produced by MDMA (Schmidt et al., in press). To ensure that the observed effect of chloral hydrate was not due to an anesthetic-induced hypothermia, an additional experiment was conducted using isothermal pads heated to 37°C to avoid any drop in body temperature in the anesthetized rats. Under these conditions, the normal hyperthermic response to MDMA was observed in both saline- and chloral hydrate-pretreated rats (fig. 2B). However, this manipulation did not alter the protective effect of the anesthetic as measured 1 week later (fig. 2A).

In contrast to the effect of chloral hydrate anesthesia at the 1 week time point, the acute depletion of 5-HT produced by MDMA at 3 h was similar in control and anesthetized rats. Changes in the level of striatal monoamines and their metabolites are given in table 1. Chloral hydrate anesthesia did not alter the MDMA-induced depression of 5-HT concentrations in the striatum nor in the cortex or hippocampus (not shown). Similarly, the MDMA-induced changes in the concentrations of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were not significantly altered in the anesthetized animals.

4. Discussion

The results show that chloral hydrate anesthesia interferes with the development of MDMA-induced neurotoxicity while not altering the acute effect of MDMA on serotonergic neurons. This suggests the immediate delivery of MDMA to the brain is not simply decreased in anesthetized animals. The similar effect of MDMA on striatal concentrations of dopamine and DOPAC in both control and chloral hydrate-treated rats also suggests the distribution of MDMA is not altered by the anesthetic. The protective effect of the anesthetic cannot be explained through an effect on body temperature. Hypothermia has been shown to attenuate several forms of neurotoxicity including that due to MDMA (Schmidt et al., in press). However, even in the absence of an anesthetic-induced hypothermia, chloral hydrate reduced the long-term serotonergic deficits produced by MDMA. A possible explanation of these results can be derived from a comparison of the two in vivo voltammetric studies described earlier. The failure of Gazzara et al. (1989) to observe an increase in striatal dopamine release with MDMA contrasts with the report by Yamamoto and Spanos (1988) of large increases in dopamine release in awake animals under otherwise identical conditions. Interestingly, an earlier study by Spanos and Yamamoto (1986) using urethane-anesthetized rats also demonstrated a slight decrease in dopamine release following MDMA administration. An attenuation of MDMA-induced dopamine release by chloral hydrate is compatible with these results and our own, since disrupting dopamine release with agents such as α-methyl-p-tyrosine has been demonstrated to antagonize MDMA-induced neurotoxicity (Stone et al., 1988; Schmidt et al., 1990).

One observation which may be inconsistent with

this interpretation is the acute MDMA-induced increase in dopamine concentrations in both control and chloral hydrate-anesthetized rats. This effect is blocked by nomifensine indicating it occurs in response to the carrier-mediated efflux of dopamine from the nerve terminal (Schmidt et al., submitted). Thus, the increase in dopamine concentrations would tend to suggest that transmitter release is occurring in chloral hydrate-anesthetized rats inspite of in vivo release data to the contrary. Although the basis of this discrepancy is not presently known, there are conditions under which increases in dopamine synthesis and concentration do occur in the absence of transmitter release. One well-described example of this is the increase in dopamine concentrations observed following the administration of γ-butyro-lactone to rats (Jennewein et al., 1986).

Although the effect of chloral hydrate anesthesia on amphetamine-induced dopamine release has not been specifically studied, several investigations have described interactions between chloral hydrate and drugs altering chloral hydrate dopaminergic function. Ford and Marsden (1986) reported chloral hydrate markedly attenuated the increase in striatal DOPAC concentrations observed in vivo following the administration of haloperidol. Others have reported that the electrophysiological response of midbrain dopaminergic neurons to amphetamine is altered by chloral hydrate (Kelland et al., 1989) as are some post-synaptic responses to dopamine (Clemens and Phebus, 1983).

In conclusion, our results indicate that chloral hydrate anesthesia can block the development of MDMA-induced neurotoxicity. Although further work is required to clarify the effect of chloral hydrate on MDMA-induced dopamine release, these results are consistent with the hypothesis that dopamine release plays an obligatory role in the neurotoxicity of MDMA. The results also suggest a need for caution in interpreting the results of neurophysiological studies on MDMA and related agents when such studies are conducted on chloral hydrate-anesthetized animals.

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