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Tolerance to the hypothermic effects of Δ^9-tetrahydrocannabinol as a function of age in the chicken

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Summary

1. (−)-Δ^9-Tetrahydrocannabinol was administered to chicks (*Gallus domesticus*) of various ages and body temperature and tolerance to the hypothermic effects of the drug were examined as a function of age.

2. The hypothermic effects were found to be greater in neonatal than older animals and likewise tolerance took longer to develop in younger chicks.

3. The rate of tolerance development, however, could be increased in the neonate by preadministration of a high dose of the drug.

Introduction

Age has been shown to be an important determinant of drug effects in animals (cf. Sereni & Puncipi, 1968). In general, neonates tend to be more sensitive than are adults to the same dose of drug. For example, morphine tends to be about 4 times more potent in 14 day old rats than in rats that are 28 days of age (Kupferberg & Way, 1963). Similarly, the LD50 of newborn rats to pentobarbital is about one-third that of adult rats (Bianchine & Ferguson, 1967).

With respect to the effects of drugs on body temperature, the same drugs may increase or decrease body temperature depending on the age of the animal. For instance, 1 mg/kg of chlorpromazine elevates the body temperature of 10 day old mice but depresses body temperature in 38 day old mice (Bagdon & Mann, 1962). The explanation for these differences in drug action has generally been that drugs tend to accumulate in the body fluids of young animals compared with adult animals because of a decreased rate of metabolism in the young, and/or that drugs penetrate into the brains of young animals to a much greater extent, owing to their blood-brain barriers being less well developed than in older animals (Sereni & Puncipi, 1968).

There is a great deal of evidence indicating that the blood-brain barrier of the chick is not fully developed until about 3 weeks after hatching (Lajtha, 1957). For instance, Spooner, Winters & Mandell (1966) have reported that 93% of ^{3}H-noradrenaline penetrates into the brain of the day old chick within two minutes after parenteral injection, compared with only 8% ^{3}H-noradrenaline penetration into the brain of the 30 day old chick. These considerations led us to investigate dose-effect relations between (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC) and hypothermia, as well as tolerance to these effects in young chickens.

Methods

The subjects in the first experiment were 140 Vantress cockerel chicks obtained from a commercial hatchery and housed in a brooder maintained at 35° C until the appropriate age for the experiment. Groups of birds were tested at 10 hours, 14 days, or 25 days of age at an ambient temperature of $22{\cdot}3 \pm 0{\cdot}5$° C. There were 50 birds in each of the first two groups ($n=10$ per subgroup) and 40 in the last group. The groups were further subdivided and given an intramuscular injection of dimethyl sulphoxide (DMSO), 1·0, 10·0, 31·6, or 100·0 mg/kg Δ^9-THC in DMSO into the breast muscle (volume=1 ml/kg). Control and drug-treated chicks were kept in groups of ten throughout the experiment. Rectal temperatures were determined immediately prior to injection and then at 1, 2 and 4 h after injection with a Yellow-Springs Telethermometer (Model No. 34 TD) with a pliable rectal probe. Average decreases in body temperature for each group of subjects were determined for each of these time periods.

In our second experiment we compared the rate at which chicks of different ages developed tolerance to the hypothermic effects of 10·0 mg/kg Δ^9-THC. The subjects were obtained from the same source as in the previous experiment and all subjects were housed in the brooder between experimental sessions. Beginning 12 h after hatching, 17 subjects received daily injections of either DMSO ($n=10$), or 10·0 mg/kg Δ^9-THC ($n=7$). Injections of Δ^9-THC were continued until Δ^9-THC no longer induced a change in body temperature different from that noted in control animals receiving only DMSO. Body temperature was measured immediately prior to injection and then two hours later. Animals in the older age group received injections commencing when they were 15 days of age.

Results

Changes in body temperature as a function of age

The average changes in body temperature as a function of age and dosage are presented in Figure 1. In the 10-hour old chicks decreases in body temperature tended to be proportional to dosage at doses of 10·0 mg/kg and above. Most of the temperature decreases occurred during the first hour after injection, after which the body temperature remained relatively stable. At four hours post injection, the mean body temperatures (° C) and standard errors (S.E.) of the placebo, 1·0, 10·0, 31·6, and 100·0 mg/kg groups were $39{\cdot}4 \pm 0{\cdot}23$; $39{\cdot}16 \pm 0{\cdot}15$; $34{\cdot}58 \pm 0{\cdot}23$; $33{\cdot}49 \pm 0{\cdot}41$ and $32{\cdot}95 \pm 0{\cdot}14$ respectively.

At 14 days of age, doses of 10·0 mg/kg Δ^9-THC and above also produced a decrease in body temperature, but the decreases were only about half as large as those observed in the 10-hour old chicks. The onset of the hypothermic effect was also slower in the 14 day old chicks with the gradual drop in body temperature not levelling off until 2 hours after injection. The 10·0 mg/kg dose of Δ^9-THC produced decreases in body temperature about as large as those seen at 31·6 and 100·0 mg/kg. At four hours, body temperature for each of the five groups was $42{\cdot}99 \pm 0{\cdot}10$; $42{\cdot}34 \pm 0{\cdot}11$; $39{\cdot}95 \pm 0{\cdot}33$; $39{\cdot}50 \pm 0{\cdot}31$ and $39{\cdot}70 \pm 0{\cdot}28$ respectively as dosage increased.

At 25 days of age, doses of 10·0 mg/kg Δ^9-THC and above still tended to produce decreases in body temperature, but the decreases were even smaller. Again. the

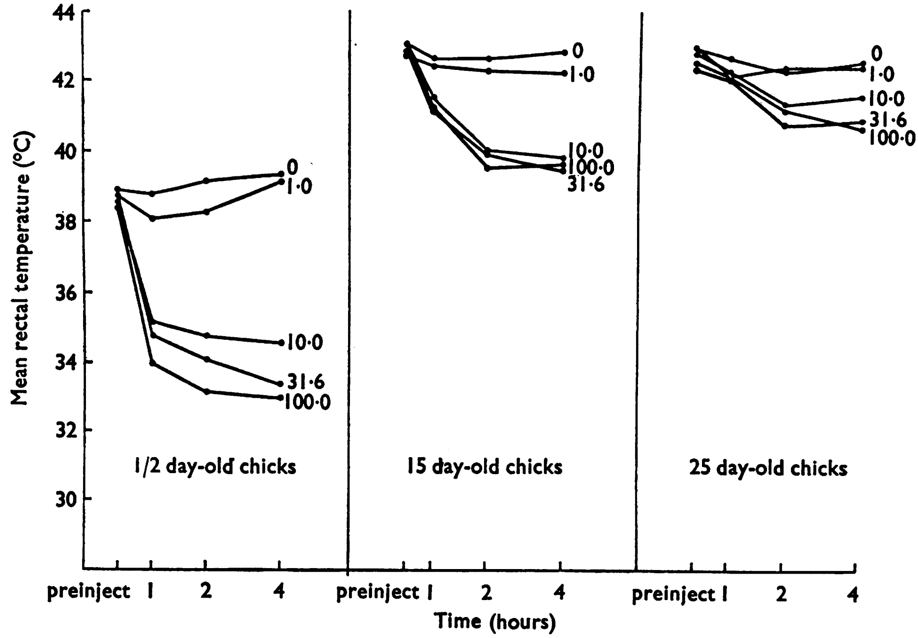

FIG. 1. Effects of intramuscular injections of Δ^9-tetrahydrocannabinol on 10 hour, 15 day- and 25 day-old chicks. (Room temperature=22·3±0·5° C).

onset of the hypothermic effect was slower than in the 10-hour old chicks. The new body temperatures for each of the five groups at four hours after injection was 42·56±0·14 ; 42·40±0·31 ; 41·56±0·24 ; 40·90±0·36 and 40·66±0·28 respectively as dosage increased.

The overall pattern of results indicates that the body temperature was lowered to a much greater extent in the younger animals than in the older animals after the same mg/kg dose of drug. The data also suggest that the onset of the hypothermic effect is more rapid in the younger birds, since the greatest decreases in body temperature tended to occur 2 h after injection in the older birds and within 1 h in the younger birds. These effects are consistent with the notion that Δ^9-THC is penetrating into the brains of the younger chicks more rapidly and to a greater extent than in the older animals.

Development of tolerance as a function of age

The results of the tolerance study are presented in Table 1. The Table shows that in the older animals, tolerance to the hypothermic effect of Δ^9-THC begins to appear as early as the second injection and body temperature does not differ from that of control chicks by the third injection. With younger animals, however, tolerance appears to develop more slowly. Little tolerance is apparent until after the fourth injection and the effects of 10·0 mg/kg Δ^9-THC on body temperature do not disappear until after the 14th injection.

These effects may be related to the maturational state of the animal. In the birds that had not received Δ^9-THC previously, an initial dose of 10·0 mg/kg decreased body temperature from 43·1° C to 41·8° C for birds 21 days of age, from 42·6° C to 40·3° C in birds 15 days of age, and from 39·8° C to 35·5° C in birds

TABLE 1. *Effects of chronic administration of* 10 *mg/kg Δ^9-tetrahydrocannabinol, once daily on body temperature in 1–2 and 2–3 week old chicks*

No. of doses	Age (days)	Changes in body temp. (°C) Control (n=10)	Drug (n=7)	No. of doses	Age (days)	Changes in body temp. (°C) Control (n=10)	Drug (n=20)
1	1	−0·1	−4·3*	1	15	−0·4	−2·3*
2	2	−2·0	−3·9*	2	16	−0·2	−1·2*
3	3	0	−4·9*	3	17	−0·8	−0·3
4	4	−0·3	−3·6*	7	21	−1·3*	−0·7
7	7	−0·1	−0·9*				
8	8	−0·2	−0·6*				
11	11	+0·1	−0·3*				
14	14	−0·3	−0·1				
15	15	−2·7*	−0·2				

* $P<0·05$ (Mann Whitney U test). All subjects received 10 mg/kg Δ^9-THC on day 15 and 7 for the 1–2 and 2–3 week old animals respectively. Rectal temperature was recorded 2 h after intramuscular injection (volume=1 cc/kg).

12 hours old. The greater drop in body temperature after Δ^9-THC in young chicks might result from greater amounts of Δ^9-THC penetrating into the brains of the younger animals due to the immature state of their blood-brain barriers (Lajtha, 1957 ; cf. Allen & Marley, 1967). The apparently slower development of tolerance in the younger birds may be related to either more rapid onset and larger effect of the 10·0 mg/kg dose in these birds, the difference in pretreatment baseline body temperatures which change as a function of age, or both of these factors.

Since tolerance does not begin to appear in chicks until they are approximately four days of age, it appears that a maturational factor may be involved in tolerance to Δ^9-THC. However, we have previously observed (unpublished observations) that following large dosages of Δ^9-THC in neonatal chicks, these animals are less affected by subsequent injections of lesser dosages of this drug than are animals that have not received Δ^9-THC previously. On the basis of this observation, we conducted the following experiment to determine whether tolerance would occur to a single dose of Δ^9-THC in the day old chick.

Twelve hours after hatching, five chicks were injected i.m. with 180 mg/kg Δ^9-THC in DMSO, while five other chicks were injected with the vehicle only. The following day, all subjects were given a single intramuscular injection of 10·0 mg/kg Δ^9-THC. Rectal temperature was determined immediately prior to injection and at two hours after injection. The mean change and standard error in body temperature produced by 10·0 mg/kg Δ^9-THC for subjects receiving 180 mg/kg Δ^9-THC 12 h after hatching was $0·0 \pm 0·7°$ C while control subjects that had not received Δ^9-THC previously showed a 5·2° C decrease ($\pm 0·5°$ C) in body temperature. This difference was significant using the Mann Whitney U statistic (Siegel, 1956) (U=0, $P<0·01$).

Discussion

We have proposed that the greater responsiveness of the 10-hour-old chicks to the drug in experiment one is a result of the relative immaturity of their blood-brain barriers.

The increased sensitivity of the younger animals could, however, be due to several other factors. For instance, chicks are much more vulnerable than older animals to decreases in heat production because of their poor thermal insulation (Marley & Stephenson, 1970). A greater density of down and feathers, or pilo-erection of feathers by older animals would thus offer them greater insulation than would be possible in the case of younger chicks, should the capacity for heat production be impaired. This is especially important because of the higher surface/volume ratio of the young relative to the older animals. In addition to an increase in the density of down and feathers, the greater size of the older animals is accompanied by a decrease in their surface/body volume ratio. This means that the metabolic rate of the older subjects ought to be lower than that of the younger chicks (cf. Lasiewski & Dawson, 1967), and therefore they might be expected to show greater decreases in body temperature at any given ambient temperature below their thermoneutral ranges (cf. Barratt & Pringle, 1941).

Secondly, since thermoregulatory mechanisms in the newly hatched chicks are also immature, young chicks exhibit a slight poikilothermia such that their body temperature is much more dependent upon the ambient room temperature than is the case with older animals (Wekstein & Zolman, 1967). Since the thermoneutral range for the day old chick (approx. 35° C) differs considerably from that of the 14-day-old (31–35° C) or 28-day-old chicks (26–31° C) (Freeman, 1963), one might also argue that as a result of their higher thermoneutral range, the ambient room temperature constituted a greater stress for them compared to the other two age groups. Since the extent of the variation between the thermoneutral range was chiefly related to the age parameter, this could also account for the greater sensitivity of the younger animals.

These results indicate that the mechanisms responsible for the development of tolerance to Δ^9-THC are functional within hours of hatching in the chick. Since tolerance did not develop so rapidly with a lower initial dose (10·0 mg/kg), these experiments also indicate that the development tolerance is a function of both the age of the animal and the drug dose.

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