Effect of 7,12-Dimethylbenz (α) anthracene upon Adrenal Cortex of Mammals

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In rats of Sprague-Dawley strain 7,12-Dimethylbenz (α) anthracene (DMBA) causes massive and selective necrosis with apoplexy within the inner zones of adrenal cortex¹⁾²⁾³⁾. The adrenal apoplexy induced by DMBA was found to be related to corticosterone content of adrenal gland by the authors⁴⁾. Species-difference in adrenocortical secretion was reported by Bush⁵⁾ and Holzbauer⁶⁾. In the present experiments the strain-difference of rats in adrenal apoplexy was abolished by corticotropin-pretreatment. In the other species of mammals than rats, however, no adrenal necrosis was induced even by the lethal dosis of DMBA, and ACTH could not elevate the adrenals of them to the susceptible state wherein DMBA induced necrosis and apoplexy in the cortex.

Materials and methods

Our sample of DMBA, m.p. 122—123°C, was purified by Florisil chromatography: the purified compound ran as 1 spot in thin layer chromatography. In some experiments DMBA was dissolved in sesame oil and administered on a single occasion by intragastric instillation by means of a soft rubber catheter. Others were injected in a vein with a fine emulsion of DMBA. One ml of the emulsion contains DMBA, 5 mg. The corticotropin was a commercial preparation. Subcutaneous injections of ACTH, daily for 7 days, were performed before the administration of DMBA.

All female animals of some strains of mice (C_3H/HeJ , $C_{57}B1/6J$, dd0 and na₂), some strains of rats (Sprague-Dawley, Long-Evans, Wistar and Boston), hamster, guinea pig, rabbit, cat and dog were sacrificed after the administration of DMBA. The adrenals were inspected, weighed and sectioned for histological examinations. Hematoxylin-eosin stain and Masson's trichrome stain were performed.

Results

(1) Influence of strain of rats upon adrenal injury

Adrenal necrosis and apoplexy induced by DMBA has always been observed in Sprague-Dawley strain of rats. The adrenal glands in which apoplexy has been induced by DMBA

have an apparent appearance. The glands are dark red in color, turgid with blood and approximately double the weight of adrenals of undamaged controls. No extravasation of blood in the edematous tissue surrounding the adrenals was observed. Microscopy revealed complete necrosis of zona fasciculata and reticularis with much more hemorrhage around the necrotic area. Zona glomerulosa and adrenal medulla, however, appeared undamaged.

Neither necrosis nor apoplexy could be detected in the adrenals of some rats in the strains of Wistar, Long-Evans or Boston. ACTH, 8 units daily for 7 days, changed all adrenals of these strains to be susceptible to DMBA. The hydrocarbon was fed on the 7th day of ACTH-treatment, and autopsy was on the 10th day. After the feeding of DMBA, all of the ACTH-treated rats developed adrenal hemorrhage (Table 1).

Table 1. Strain difference of rat

A single meal of DMBA, 30 mg, was given to mature female rats after the daily subcutaneous injection of ACTH, 8 units for 7 days

Strain of rat	No. of rat	ACTH-pretreatment	Adrenal apoplexy
Sprague-Dawley	10	+	10
	10	—	10
Long-Evans	10	—	3
	10	—	3
Wistar	5	+	5
	5	—	1
Boston	5	+	5
	5	—	0

In rats, sacrificed 7 months after the feeding, adrenal calcification was detected, but no adrenal tumor appeared.

(2) Influence of species of mammals on effect of DMBA upon adrenal gland

(a) Mice

In each 20 young mice of the strain of C₃H, C₅₇Black, dd, and na₂, adrenal necrosis and apoplexy was induced neither by a single feeding of DMBA, 10mg, nor by a single injection in a caudal vein of DMBA, 5 mg. Corticotropin, 5 units daily for 8 days before the administration of DMBA, could not change the adrenals of 15 dd-mice to be susceptible to DMBA. All of 20 C₃H-mice, given by 20 or 30 mg of DMBA orally, died 3-7 days after. But neither necrosis nor hemorrhage were observed in the adrenal cortex of the died mice. After more than half year single feeding of DMBA, 10 mg, produced both many ovarian tumors (incidence: 13/22, mainly granulosa cell type) in C₃H-mice and some splenohepatomegalies (incidence: 10/17, mainly lymphocytic leukemia) in C₅₇Black-mice. Three of C₅₇Black-mice died of thymoma in the course of 7 months after the feeding. No adrenal calcification

was found in their autopsies, although half of Sprague-Dawley rats bearing mammary tumor induced by DMBA had adrenal calcification. One adrenal tumor (cortical cell adenoma) was detected in 6 survival C₃H-mice, injected with 5 mg of DMBA, 7 months after the injection.

(b) Hamsters

Ten young hamsters, injected by 5 mg of DMBA intravenously, were sacrificed 3 days later. Five adults, fed by 75–100 mg of DMBA, died 3–5 days after. Neither necrosis nor bleeding were observed in adrenals of these hamsters. Ten hamsters, fed with 30 mg of DMBA, had no any tumors in the course of 7 months after the feeding.

(c) Guinea pigs

Single feeding of DMBA, 200 mg, caused leucopenia and petechia in the gastrointestinal mucosa in 22 mature guinea pigs and was followed by deaths of all animals in the following 1–2 weeks. No hemorrhagic necrosis, however, was detected in their adrenal cortex.

Half of 14 young guinea pigs, fed by 50 mg of DMBA, were survival 7 months after the feeding, but neither adrenal tumor nor adrenal calcification were detected. Pretreatment of ACTH, 8 units daily for 7 days, could not alter the susceptibility of the adrenal gland to DMBA.

(d) Rabbits

Ten rabbits, 1,200–1,500 g of body weight, were injected intravenously by 12.5–25 mg of DMBA, respectively. No remarkable alterations were found hematologically or histologically during the following 7 days. Six rabbits, weighed 2,000 g or more, were fed with 0.5–1 g of DMBA. Some of them died of severe diarrhea and hinderness of the hemato-poietic system, but no adrenal apoplexy was observed in their autopsies. Survival of them had neither tumor nor calcification in the adrenal gland 7 months later.

(e) Cats

Intravenous injection of 100 mg of DMBA could not induce adrenal necrosis and apoplexy in 5 young cats, even after the injection of ACTH, 16 units daily for 1 week.

(f) Dogs

Ten young dogs were fed with 0.5–1 g of DMBA or injected in a femoral vein by 100 mg of emulsified DMBA. No adrenal necrosis was found within 3–7 days after the administration.

Discussions

In the species of rats DMBA causes necrosis and apoplexy in the adrenal cortex selectively. Death of adrenocortical cells involved only fascicular and reticular zones of the

adrenal cortex, whereas zona glomerulosa and adrenal medulla are spared from injury. Moreover, hemorrhage occurs in no organ other than adrenal cortex. Adrenal apoplexy induced with DMBA is hormone-dependent in character. It occurred in every female rat aged 50 days, and in no rat aged 25 days. Furthermore, DMBA induced adrenal hemorrhage in all rats aged 50 days during the first week after removal of the pituitary. The administration of corticotropin to juvenile rats, or to adults deprived of the hypophysis three weeks earlier, converted the adrenal cortex to a state susceptible to damage when DMBA was given: this susceptible state is directly related to the content of corticosterone in the adrenal ⁴⁾. ACTH should accelerate production of corticosterone in adrenals, and might abolish the strain-difference of rats in the way. It has been postulated that the cause of injury is related to the similarity of DMBA and corticosterone¹⁾⁴⁾. It was of interest that a single feeding of any of a number of polycyclic aromatic hydrocarbons or aromatic amines in rats prevented adrenal injury from DMBA^{7)~9)}.

In other than the species of rats adrenal necrosis and apoplexy was induced neither by the lethal dosis of DMBA nor by the pretreatment of corticotropin. Explanation of the species-specific nature of adrenal injury by DMBA presented the greatest difficulty as well as with DDD. If hypophysectomy inhibited the effect of DMBA, it might be argued that effect is mediated through a species-specific structural composition of ACTH. However, this cannot be considered because necrosis and apoplexy occurs in adrenals of the hypophysectomized rats, and because ACTH from other than rat elevates adrenals of the rat to the susceptible state to DMBA. The explanation will probably be found at the adrenal cellular level, especially adrenocortical hormones. Species-difference in the adrenocortical hormones was studied paperchromatographically by Bush⁵⁾ and Holzbauer⁶⁾. Compound B, namely corticosterone proved in many species, but the adrenals of the other than rats have less amounts of compound B than rats. Rats have hardly compound F, namely hydrocortisone in the adrenal tissue. The ratio of F/B was less than 0.05 in rats, otherwise it was 0.1 in rabbits, 5—6 in dogs and 20 in monkeys. DMBA might combine strongly only with something like corticosterone-producer in the adrenocortical cells owing to the stereochemical similarity between both. The corticosterone-producer might be species-specific nature.

Conclusions

Strain-difference of rats in adrenal necrosis and apoplexy induced by DMBA was abolished with corticotropin-pretreatment. In the species of mice, guinea pigs, hamsters, rabbits, cats and dogs, no adrenal necrosis and apoplexy was induced even by the lethal dosis of DMBA. Corticotropin-pretreatment could not convert adrenals to be susceptible of DMBA in some species of mammals.

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哺乳動物副腎皮質に対する 7, 12-Dimethylbenz (α) anthracene の効果

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DMBA によるラット副腎皮質傷害の発現率はラットの系統差により変動するが, ACTH前処置によりその差は消失し, 全例に傷害が発生した。

ラット以外の数種哺乳類 (マウス, モルモット, ハムスター, ウサギ, ネコ及びイヌ) における DMBA の副腎皮質に及ぼす効果を検索したが, 致死量でも又は, ACTH 処置後でもすべてに壊死を発生させることができなかった。