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*Brief Communication*

# 7,12-Dimethylbenz(a)anthracene-induced Adrenocortical Necrosis and Its Inhibition by Metopirone (SU4885)[1]

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*Summary.* Subcutaneous injection of 40 mg of Metopirone ditartrate in aqueous solution inhibited the induction of adrenocortical necrosis by 5 mg of 7,12-dimethylbenz(a)anthracene (DMBA), given intravenously as an oil emulsion 30 minutes before or after the Metopirone. No such inhibition was observed when the interval between injections of the agents was one hour or more. Two doses of 2.5 mg of DMBA emulsion injected simultaneously or not more than 30 minutes apart induced adrenocortical necrosis, but these doses were ineffective when there was one hour or more between them.

*Introduction.* 7,12-Dimethylbenz(a)anthracene (DMBA), one of the most potent chemical carcinogens, has been shown to induce massive acute necrosis of the fasciculate zone of the adrenal cortex in rats (4, 5). Although many other species have been treated in large numbers with comparable doses of DMBA, a similar adrenal lesion has not been noted in any of them (2).

Normal liver function is necessary for DMBA itself to act as the necrotizing agent (7), but it has been shown that 7-hydroxymethyl-12-methylbenz(a)anthracene, which is more active than the parent hydrocarbon (1, 7), is independent of normal liver function, suggesting that it is the 7-hydroxy metabolite, formed in the liver, which is the active agent in the adrenal.

Adrenal necrosis is not induced by DMBA in the hypophysectomized rat, but such pituitary-deficient animals are rendered susceptible by treatment with adrenocorticotropic hormone (ACTH) before dosage with DMBA (6). The correlation of susceptibility to DMBA with a certain level of adrenal corticosterone led to the suggestion that the necrotizing action of DMBA was dependent on the presence of active steroid synthesis.

Currie *et al.* demonstrated that Metopirone (SU4885, 2-methyl-1,2-bis(3-pyridyl)-1-propanone), which is a specific inhibitor of adrenal function, also inhibited the necrotizing action of both DMBA (3) and 7-hydroxymethyl-12-methylbenz(a)anthracene and so presumably acted directly on the adrenal cortex. They assumed that this inhibition was related to the fact that Metopirone can be shown to inhibit 11-hydroxylation in some species. In the experiments of Currie and his colleagues, and others who have repeated his observations, pretreatment of rats with Metopirone, before giving DMBA, has been prolonged, the inhibitor usually being administered in oil over a period of 16 hours or more. Such a mode of administration, if essential, would be in keeping with the concept that the inhibitory activity was related to the compound's ability to interfere with 11-hydroxylation.

The following experiments were intended to determine the relation in time which is necessary between Metopirone and DMBA administration if inhibition of necrosis is to be effected. Other experiments were designed to find out the rapidity with which DMBA is inactivated after injection and therefore a measure of the time within which it must interact with the adrenal cortex.

*Materials and Methods.* Male rats of an inbred hooded strain weighing from 165 to 190 gm were used in all experiments.

In the first series of experiments, 40 mg of Metopirone as its ditartrate[2] in aqueous solution at a concentration of 100 mg/ml was injected subcutaneously at intervals (Table 1) before or after the injection into the left femoral vein of 1 ml of an oil emulsion containing 5 mg of DMBA.[3]

In the second series of experiments, rats were given 30 mg of ethionine in aqueous solution i.p. half an hour before 2.5 mg of DMBA in emulsion were injected intravenously. A second intravenous injection of 2.5 mg DMBA was given after intervals varying between 5 minutes and 17 hours (Table 2). There were additional injections of 30 mg of ethionine at 4-hour intervals, to maintain inhibition of enzyme induction until the second dose of DMBA was completed.

All rats were killed 72 hours after DMBA injection. Necrosis of adrenocortical cells was assessed microscopically on sections stained with hematoxylin and eosin. Marked congestion or hemorrhage, which may have been due to DMBA administration and could not be distinguished macroscopically from necrosis, was differentiated microscopically and was not taken as evidence of adrenocorticolytic activity.

*Results.* Necrosis of the adrenocortical fasciculata cells occurred in 76% of rats injected with DMBA alone (Table 1) and also in rats injected with 40 mg of aqueous Metopirone

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**Table 1**

| DMBA alone | Time in minutes at which Metopirone was injected relative to DMBA | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | −120 | −60 | −30 | −5 | +5 | +15 | +30 | +60 | +120 |
| 32/42 | 5/5 | 4/5 | 0/5 | 0/19 | 0/9 | 0/5 | 0/5 | 4/9 | 5/5 |

Incidence of adrenal necrosis in male hooded rats injected intravenously with 5 mg of dimethylbenz(a)anthracene (DMBA) in oil emulsion and subcutaneously with 40 mg Metopirone ditartrate. −, before DMBA; +, after DMBA.

**Table 2**

| 0 | Interval between doses | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 min | 15 min | 30 min | 1 hr | 2 hr | 5 hr | 17 hr |
| 14/16 | 3/5 | 2/5 | 1/5 | 0/5 | 0/5 | 0/5 | 0/5 |

Incidence of adrenal necrosis in male hooded rats injected intravenously with 2 doses of 2.5 mg dimethylbenz(a)anthracene in oil emulsion with varying intervals between the doses.

ditartrate one hour or more before or one hour or more after the injection of the DMBA. No adrenal necrosis was seen among 43 rats which were given subcutaneous injections of the Metopirone solution at various times between 30 minutes before and 30 minutes after the injection of DMBA.

Twenty mg of Metopirone ditartrate injected in aqueous solution at any time in relation to DMBA did not inhibit the induction of adrenal necrosis. These experiments suggest that inhibition of the necrotizing action of DMBA by Metopirone is a simple competitive inhibition dependent on the relative concentrations of the compounds. The very time-limited action of Metopirone ditartrate, as opposed to the free base (3), is presumably due to its rapid absorption and metabolism. The oil-soluble base might be expected to be absorbed more slowly and so act over a longer period.

The ability of Metopirone to inhibit the action of DMBA, even when administered up to 30 minutes after the carcinogen, could mean either that it displaces DMBA from its site of action in the cell or, more probably, that at least 30 minutes is required for the metabolism of DMBA to its effective form and its localization in the adrenal cortex.

In the second series of experiments (Table 2) it was necessary for the second injection of 2.5 mg of DMBA to be injected within 30 minutes to induce adrenal necrosis. At longer intervals there was no necrosis in the 20 rats treated. This is further illustration that the necrotizing action of DMBA is dependent on the achievement of a critical concentration. Delay in the injection of the second of the two 2.5-mg doses of the compound will allow metabolism and removal of a significant portion of the initial injection so that a threshold dose is not achieved. There is a similarity between the critical time, relative to DMBA, for achieving inhibition by the injection of Metopirone and the critical time in which divided doses of DMBA must be administered to be effective.

The data are in keeping with the concept that DMBA attaches to receptors already present in the adrenal cortex, but that to do this it must achieve a minimum concentration, either to overcome competition from other factors, or to make the attachment reaction proceed. Metopirone presumably has an affinity for the same receptors and inhibits the action of DMBA by blocking access to them. There is no reason to suppose that the effectiveness of Metopirone is related to its ability to inhibit steroid hydroxylation reactions.

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