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EFFECTS OF 6-METHYL-17-ACETOXY-PROGESTERONE ON PITUITARY–ADRENAL FUNCTION*

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Abstract—Plasma corticosterone levels were lower, both before and after noxious stimuli, in female rats treated with 6-methyl-17-acetoxy-progesterone (MAP) than in nontreated control animals. Adrenals from MAP-treated animals produced less corticoid per unit weight of adrenal tissue *in vitro*, and the adrenals were much smaller than those from control animals, indicating a sizable diminution in total adrenal capacity. This is felt to be due to a curtailed ACTH stimulation *in vivo* rather than a direct effect of MAP on the adrenal cortex because incubated adrenals from MAP-treated rats responded as vigorously to ACTH as did those from untreated rats. The administration of MAP to intact rats did not significantly alter the rate of hepatic reduction of the A-ring of cortisone.

THE orally active progestational agent, 6-methyl-17-acetoxy-progesterone (MAP), has been shown to produce severe adrenal atrophy in rats with an intact pituitary gland.[1] Associated with the atrophy was a depletion of pituitary ACTH concentration and inhibition of ACTH release in response to acute stress.[2] Certain data in the latter study indicate that, in hypophysectomized rats, MAP may induce adrenal atrophy, in part at least, by a direct action on the adrenal cortex. Some disagreement therefore exists as to whether MAP influences the adrenal only through the pituitary or has, in addition, a direct effect on the adrenal.

Indirect evidence that MAP depresses adrenal function as well as adrenal size was obtained by Edgren *et al.*[3] when they demonstrated that cold shock killed 4 of 7 mice that had received daily injections of 500 μg MAP for 2 weeks but killed none of 10 control animals.

With the aim of further elucidating the mechanism by which MAP influences pituitary–adrenal function, we have studied the effects of this compound on plasma corticosterone levels in stressed rats, corticoid production by adrenals *in vitro* obtained from MAP-treated rats, and the effect of MAP on hepatic reduction of the Δ4–3 ketone group of corticoids.

METHODS

Adult female rats (200–250 g) from the Holtzman strain were used throughout these studies.

Plasma corticosterone. Blood samples were obtained from rats under pentobarbital anesthesia (4 mg/100 g body weight). Serial samples were taken[4] from each rat,

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beginning 20 min after the intraperitoneal injection of sodium pentobarbital, and free plasma corticosterone was determined fluorometrically by the method of Silber *et al.*[5] MAP was administered subcutaneously in sesame oil, 1 mg/day for 10 days. Plasma concentrations of MAP greater than could reasonably be expected to occur (up to 100 μg/ml) in these experiments did not interfere with the determination of corticosterone.

Incubated adrenal glands. 5 mg of a depot preparation of MAP was administered intramuscularly to each rat 14 and 7 days prior to sacrifice by severance of the cervical vessels. The adrenals were rapidly removed and studied for corticoid production *in vitro* as previously described.[6]

Hepatic reduction of A-ring. Liver homogenates, prepared from MAP-treated and control animals, were tested for the ability to reduce the A-ring of cortisone as described previously,[7] except that the reaction was carried out for 30 min instead of 2 hr. Two preparations of MAP were employed: one dissolved in sesame oil and administered subcutaneously, 1 mg/day for 10 days; the other, a depot preparation, was given intramuscularly, 5 mg on days 14 and 7 prior to sacrifice of the animals. The data (decrease in adrenal weight and rate of A-ring reduction) were not different in these two groups of animals and are therefore combined in Table 3.

RESULTS AND DISCUSSION

It has been shown previously[4] that pentobarbital will block ACTH release in the resting state but not in rats from which serial blood samples are taken by excising the tip of the tail. Animals subjected to this procedure exhibit a rise in plasma corticosterone concentration while under pentobarbital anesthesia. This is also shown in

TABLE 1. EFFECT OF 6-METHYL-17-ACETOXY-PROGESTERONE (MAP) ON PLASMA CORTICOSTERONE LEVELS IN ACUTELY STRESSED FEMALE RATS

Drugs administered	Plasma corticosterone, μg/100 ml, at intervals after pentobarbital administration			
	20 min	35 min	50 min	80 min
Pentobarbital n = 10	34·8 ± 3·8	28·8 ± 3·6	45·6 ± 6·7	59·9 ± 6·4
Pentobarbital to rats pretreated with MAP n = 4	13·7 ± 1·4 P < 0·02	12·5 ± 0·3 P < 0·02	14·0 ± 1·9 P < 0·02	28·8 ± 4·0 P < 0·02

Values are means ± standard error.

Table 1 of the present report together with data from female rats treated with MAP. Plasma corticosterone levels in the latter group were significantly lower than in the control group at every interval studied throughout the 80-min period of blood sampling. This depressed pituitary–adrenal response to cutting of the tail and blood loss could be due to an action of MAP on the pituitary or on the adrenal cortex or simultaneously on both of these glands.

To assess the autonomous steroidogenesis of adrenal glands exposed to MAP *in vivo*, rats were treated with this compound for two weeks, after which the adrenals were removed and incubated for corticoid production. MAP produced a moderate

but significant ($P < 0.05$) depression in steroid synthesis per unit weight of adrenal tissue (group 1, Table 2). The adrenals of these animals were also much smaller ($P < 0.001$) than those of the controls. Steroidogenesis by adrenal tissue *in vitro* has been reported[8] to reflect prior ACTH stimulation, or the lack of it, *in vivo*. The decreased steroid production by adrenals from MAP-treated animals may therefore reflect a block of pituitary ACTH synthesis and/or release rather than a direct effect

TABLE 2. EFFECT OF 6-METHYL-17-ACETOXY-PROGESTERONE (MAP) ON ADRENOCORTICOID PRODUCTION *IN VITRO*

Group	Source of adrenal tissue	Material added to incubating adrenals (unit)	Adrenal wt., mg	Corticoid production, μg/100 mg adrenal/2 hr
1	Normal females n = 7	None	57·2 ± 1·77	16·2 ± 1·40
	Females-MAP n = 7	None	36·4 ± 1·28	11·4 ± 1·01
2	Normal females n = 5	ACTH 0·1	55·4 ± 1·75	19·8 ± 0·40
	Females–MAP n = 5	ACTH 0·1	37·0 ± 1·21	18·2 ± 0·19
3	Normal females n = 4	ACTH 0·35	63·1 ± 3·33	25·1 ± 1·44
	Females–MAP n = 8	ACTH 0·35	40·8 ± 2·04	26·2 ± 1·64

Values are means ± standard error.

of MAP on the adrenal cortex. The decisive point here is whether or not MAP will modify adrenocortical responsiveness to ACTH. To answer this question, adrenals from control and MAP-treated rats were exposed to ACTH *in vitro*, in concentrations of 0·1 (group 2, Table 2) and 0·35 (group 3, Table 2) unit ACTH/1·5 ml incubation medium. Corticoid production by the adrenals of MAP-treated and control animals was of the same magnitude at both concentrations of ACTH, indicating that MAP did not diminish adrenocortical responsiveness to ACTH. This is probably the functional counterpart of the observation[1, 2] that MAP does not produce adrenal atrophy in rats when ACTH is administered concurrently. The control adrenal glands in group 3 are somewhat larger and more variable in weight than those in groups 1 and 2. Animals within a group were from the same shipment of rats but the three groups were from different shipments. This type of variation has previously been noted in this and other laboratories.

Hepatic metabolism of progesterone is blocked[1] by 6-α-methylation. Consequently, it was of interest to determine whether or not MAP would also block hepatic enzyme systems responsible for the reduction of adrenocortical steroids. If this proved to be the case, the resulting prolongation of the half-life of adrenal steroids might mask a rather severe adrenal defect. However, no experimental evidence was found for this idea. Liver homogenates from MAP-treated animals reduced the steroid A-ring at a rate that was not significantly different from the rate for control animals (Table 3).

The present studies indicate that female rats treated with MAP have an altered pituitary–adrenal response to surgery and blood loss. This can be attributed to

pituitary suppression, since the adrenals from these animals respond normally to ACTH. MAP has now been used clinically for four years with no manifest evidence of causing adrenal hypofunction, and Helmreich and Huseby[9] report that doses up to 200 mg/day for six months do not alter the plasma cortisol levels as measured

TABLE 3. EFFECT OF 6-METHYL-17-ACETOXY-PROGESTERONE (MAP) PRETREATMENT ON HEPATIC REDUCTION OF THE A-RING OF CORTISONE

Source of liver tissue	Adrenal wt., mg	Cortisone reduced, μg/200 mg liver/30 min
Normal females n = 10	55·8 ± 1·85	95·7 ± 3·10
MAP-treated females n = 10	38·4 ± 1·55 P < 0·001	88·3 ± 5·70 P > 0·2

Values are means ± standard error.

by the Nelson-Samuels method. The evidence to date does not, however, rule out the possibility of reduced pituitary reserve in women receiving MAP for long periods of time, and due caution should be exercised in exposing these patients to stresses such as ether anesthesia and major surgical procedures until this point is further investigated.

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