Effects of Sex Hormones on the Duration of Drug Action in Mice

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An altered drug response is induced in mice following pretreatment not only with repeated doses, but even after a single dose, of a sex hormone. Testosterone pretreatment produces a biphasic effect on the duration of action of hexobarbital, prolonging the action initially and shortening the action in 4–8 days after the pretreatment. The early action of testosterone appears to be associated with an effect on the hypnotic property of a drug, since both hexobarbital and barbital sleep times are prolonged while the duration of action of the muscle relaxant chlorzoxazone remains unaffected. The long-term pretreatment with testosterone leads to a shorter duration of action of drugs that are deactivated by detoxification, notably hexobarbital and chlorzoxazone, but has no effect on the duration of hypnosis produced by barbital, a drug which is predominantly eliminated unchanged.

THERE IS increasing evidence that steroids are endogenous substrates of drug-metabolizing enzymes in mammals (1–8). Such a relationship between the drug and steroid metabolism could be of great consequence for drug therapy in man. In animals the evidence for the above interrelationship consists of two kinds of data. On one hand, compounds which have been known for some time to stimulate drug-metabolizing enzymes (2, 3) have been found more recently to be effective in stimulating steroid metabolism *via* hydroxylation (1, 4–7). On the other hand, sex hormones exert a marked effect on drug-metabolizing enzymes in the rat (8, 9). This latter phenomenon is seen both as an inherent sex difference in the susceptibility to drugs (female rats sleep four times longer than male rats after the same dose of hexobarbital), and as a virtual obliteration of such a sex difference through pretreatment of male rats with estrogens or of female rats with androgens (1, 8, 9). Similar experiments with sex hormones in mice and guinea pigs have been unsuccessful (8, 9).

It seemed important to the authors that if the relationship between drug- and steroid hormone-metabolizing enzymes is applicable to mammalian species in general, sex hormones should have an effect on the duration of drug action in species other than rats, though such effects might be more subtle, and hence less readily discernible. The authors re-examined the effect in mice.

EXPERIMENTAL

Animal Treatment—Swiss Webster mice of ICR strain from Blue Spruce Farms were used for the experiments. The mice weighed 18–26 Gm. on arrival and were maintained on commercial chow diet and water *ad libitum*. Each animal was used only once. Animals were randomly assigned to either a test or the corresponding control group. Both groups were treated similarly in all respects, except that the control mice received injections of the vehicle (corn oil) in place of the hormone solution.

In the study of the effects of chronic sex hormone pretreatment the hormone in corn oil solution was administered subcutaneously every other day for a period of 1 week or longer, as stated. Female mice were thus treated either with 4 doses of 10 mg./Kg. of ethinyl estradiol in an 8-day period, or with 8 doses of 20 mg./Kg. of testosterone acetate in a 16-day period. Male mice were treated with 7 doses of 25 mg./Kg. of diethylstilbestrol in a 14-day period. Chlorzoxazone and hexobarbital assays were performed 24 hr. after the last injection of the hormone.

In the study of the development of an altered drug response due to pretreatment with only a single dose of a sex hormone, assays were carried out using chlorzoxazone,[1] hexobarbital, and barbital at 0 hr., 24 hr., 96 hr. (4 days), and 192 hr. (8 days) after the hormone pretreatment. In the special case of the 0-hr. pretreatment the hormone in corn oil was injected intraperitoneally immediately before the assay drug; in all other cases the injections were subcutaneous. In all experiments corn oil was administered to control mice on a schedule identical with that used for the corresponding test animals.

Drug Assays—The duration of drug action was assayed by means of the following drugs at the given dosages: chlorzoxazone[2] (150 mg./Kg.), hexobarbital[3] (150 mg./Kg. unless otherwise stated), barbital[4] (250 mg./Kg.). The compounds were administered as sodium salts in isotonic saline. The duration of drug effect (the chlorzoxazone prostration time and the hexobarbital or barbital sleep time) was determined by noting the time interval during which the righting reflex was lost.

Where direct comparisons were made between assays with different agents (hexobarbital, chlorzoxazone, or barbital), control and test mice were randomly selected for assay with either agent and the assays were performed contemporaneously under identical conditions. The statistical significance of the observed differences (the P value) was determined by use of the tailed t test except where the F test showed the variance ratio itself to be significant. In the latter event Wilcoxon's two-tailed Rank test was employed (10).[5]

RESULTS AND DISCUSSION

The results (Table I) show that the duration of drug action is significantly changed after chronic pretreatment of mice with an estrogen (diethylstilbestrol or ethinyl estradiol) or with an androgen (testosterone acetate). Estrogen treatment leads to the prolongation of drug action in both male and female mice; conversely, androgen treatment leads to a shorter duration of drug action.

Received September 15, 1966, from the School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214

Accepted for publication December 8, 1966.

This investigation was supported in part by the undergraduate research participation program grant GY-212 to J. A. Baker and L. L. Edelman from the National Science Foundation.

[1] The gift of chlorzoxazone from McNeil Laboratories is gratefully acknowledged.

[2] 5-Chlorobenzoxazolidone.

[3] 5-(1-Cyclohexen-1-yl)-1,5-dimethylbarbituric acid.

[4] 5,5-Diethylbarbituric acid.

[5] The authors thank Dr. P. K. Gessner for the statistical computations.

TABLE I—EFFECT OF MULTIPLE DOSE PRETREATMENT WITH SEX HORMONES ON THE DURATION OF DRUG ACTION

Sex	N[a]	Pretreatment[c] Sex Hormone	Dose, mg./Kg.	Length, Days	Duration of Drug Effect Assay Drug	Time, min.	P
F	10	Ethinyl Estradiol	10	8	Hexobarbital	60.2 ± 4.9	<0.01
F	10	None[b]			Hexobarbital	43.4 ± 3.2	
F	8	Testosterone acetate	25	16	Chlorzoxazone	50.7 ± 4.7	<0.05[d]
F	5	None[b]			Chlorzoxazone	65.7 ± 7.2	
M	5	Diethylstilbestrol	25	14	Chlorzoxazone	52.2 ± 5.0	<0.01
M	5	None[b]			Chlorzoxazone	31.6 ± 2.0	
M	7	Diethylstilbestrol	25	14	Hexobarbital	97.2 ± 13.8	<0.05
M	7	None[b]			Hexobarbital	56.5 ± 7.1	

[a] Number of animals. [b] All control animals received injections of the vehicle (corn oil) on a schedule identical to that used for the corresponding tests. [c] During the pretreatment period the indicated dose of the sex hormone was administered every 48 hr. [d] Single-tailed *t* test.

It is interesting that female mice do not appear to have an inherently greater susceptibility to hexobarbital than male mice (Table I, hexobarbital control groups). In fact, the trend appears reversed but the observed difference in the sleep times is not statistically significant ($P < 0.06$). However, a highly significant sex difference ($P < 0.001$) is apparent in the duration of action of chlorzoxazone; female mice exhibit chlorzoxazone prostration times twice as long as those of the males.

The results of the sex hormone pretreatment are amenable to two interpretations. First, it could be argued that a period of chronic hormone pretreatment is necessary for the sought effect on drug action to become manifest. Second, the possibility exists that a single administration of hormone is sufficient, but that a certain length of time has to elapse before the process initiated by this hormone injection (in effect, the first injection of a chronic pretreatment regimen) results in an observable effect. In order to distinguish between these two possibilities, the authors injected female mice with a single high dose of testosterone acetate and carried out the duration of drug effect assays after various time intervals. As shown in Table II, the authors found a significant decrease in the duration of action of chlorzoxazone and of hexobarbital 192 hr. after a single injection of a dose of either 140 mg./Kg. or 46 mg./Kg. of testosterone.

Many compounds which stimulate drug metabolism when long-term pretreatment is employed are found to be inhibitors of drug-metabolizing enzymes both *in vivo* and *in vitro* (2, 11, 12). Accordingly, such compounds are seen to have a biphasic effect on the duration of action of drugs which are subject to inactivation through rapid metabolism. In order to test whether testosterone pretreatment can elicit a biphasic effect on the duration of drug action the authors carried out a series of duration of drug effect assays immediately after the hormone administration (0 hr. pretreatment, Table II). The results show that indeed such a short-term pretreatment with either testosterone or testosterone acetate produces a prolongation of the hypnotic action of barbiturates, but that this occurs irrespective of whether the barbiturate is one which is extensively metabolized, *i.e.*, hexobarbital (13), or one which is hardly metabolized at all, *i.e.*, barbital (14). Moreover, the identical pretreatment has no effect on the duration of the paralysis produced by chlorzoxazone, a drug which is inactivated mainly by metabolism (15). These results therefore suggest that the observed longer duration of action of barbiturates is mainly unrelated to an effect of testosterone on drug-metabolizing enzymes, but rather is associated with a potentiating effect on the hypnotic action. In this latter respect, the report of Selye (16) on the anesthetic property of testosterone in partially hepatectomized rats is noteworthy.

Novick *et al.* (17) have reported that daily pretreatment of mice with testosterone for 4–5 days resulted in a prolongation of hexobarbital sleep time in these animals, whereas we found that long-term testosterone pretreatment shortens the duration of drug action (Tables I and II). In the experiments of Novick *et al.*, high levels of circulating testosterone would be expected to be present at the time of the sleep-time assay, since, in contrast to our experiments, these workers administered the last injection of the hormone only 1-hr. before the hexobarbital assay. In such circumstances testosterone could extend the duration of hexobarbital action, not only by any possible inhibition of the drug-metabolizing enzyme (Novick *et al.* report somewhat decreased microsomal metabolism in the livers of testosterone pretreated mice) but also by a direct potentiation of the hypnotic action of the barbiturate.

The authors conclude that testosterone exerts a biphasic effect on the hypnotic action of hexobarbital. The prolongation of hexobarbital sleep time in the initial period following pretreatment with the hormone appears to be mainly unrelated to an effect on drug metabolism, but instead seems to involve potentiation or synergism of the hypnotic property of the drug, as is evidenced by the fact that hypnosis due to barbital is affected similarly. On the other hand, the shorter duration of drug action after long-term pretreatment with testosterone is probably associated with a stimulation of drug-metabolizing enzymes. This is indicated by the fact that an 8-day (192 hr.) pretreatment with testosterone has no effect on the barbital sleep time, but foreshortens the duration of action of the two drugs that are extensively metabolized, hexobarbital and chlorzoxazone.

TABLE II—TIME COURSE OF DEVELOPMENT OF AN ALTERED DRUG RESPONSE IN FEMALE MICE PRETREATED WITH A SINGLE DOSE OF AN ANDROGEN

	Pretreatment			Duration of Drug Effect		
N^a	Hormone	Dose, mg./Kg.	Length, hr.	Assay Drug	Time, min.	P
9	Testrosterone acetate	140	192	Chlorzoxazone	38.4 ± 2.2	<.02
10	None[b]			Chlorzoxazone	47.4 ± 2.5	
7	Testosterone acetate	140	192	Hexobarbital	86.4 ± 8.6	<.01
9	None[b]			Hexobarbital	125.1 ± 9.4	
10	Testosterone acetate	140	192	Barbital	107.4 ± 12.7	N.S.
10	None[b]			Barbital	112.2 ± 12.1	
10	Testosterone acetate	46	192	Hexobarbital	95.2 ± 7.6	<.01
10	None[b]			Hexobarbital	138.3 ± 11.7	
10	Testosterone acetate	46	96	Chlorzoxazone	47.6 ± 3.2	N.S.
10	None[b]			Chlorzoxazone	49.4 ± 3.6	
9	Testosterone acetate	46	96	Hexobarbital	146.3 ± 7.6	N.S.
10	None[b]			Hexobarbital	162.5 ± 10.4	
10	Testosterone acetate	46	24	Chlorzoxazone	25.4 ± 3.1	N.S.
10	None[b]			Chlorzoxazone	27.4 ± 1.9	N.S.
9	Testosterone acetate	140	24	Chlorzoxazone	27.5 ± 2.4	
10	Testosterone acetate	46	24	Hexobarbital	66.2 ± 8.5[c]	N.S.
10	None[b]			Hexobarbital	69.0 ± 4.6[c]	N.S.
10	Testosterone acetate	140	24	Hexobarbital	61.2 ± 6.5[c]	
9	Testosterone acetate	140	0	Chlorzoxazone	29.5 ± 2.0	N.S.
10	None[b]			Chlorzoxazone	26.8 ± 2.3	
10	Testosterone acetate	140	0	Barbital	237.2 ± 6.9	<.01
8	None[b]			Barbital	176.5 ± 15.9	
10	Testosterone	120	0	Chlorzoxazone	27.3 ± 2.0	N.S.
10	None[b]			Chlorzoxazone	26.8 ± 2.3	
9	Testosterone	120	0	Barbital	245.8 ± 13.4	<.005
8	None[b]			Barbital	176.5 ± 15.9	
10	Testosterone acetate	280	0	Hexobarbital	76.2 ± 4.1[c]	<.005
10	None[b]			Hexobarbital	53.6 ± 4.8[c]	
10	Testosterone	240	0	Hexobarbital	94.9 ± 10.1[c]	<.01
10	None[b]			Hexobarbital	53.6 ± 4.8[c]	

[a] Number of animals. [b] All control animals received injections of the vehicle (corn oil) on a schedule identical to that used for the corresponding tests. [c] Hexobarbital was injected at a dose level of 100 mg./Kg.

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