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Drug metabolism and pharmacologic action in mice exposed to reduced barometric pressure*

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THE ENHANCEMENT or depression of drug action or metabolism or of both, induced by a variety of drugs and chemicals has been extensively investigated.[1] Similar effects are also produced by a number of stress conditions such as starvation,[2] hind-limb ligation,[3] hypobaric conditions,[4] increased or decreased temperature, and dehydration.[5]

*The research reported in this paper was conducted by personnel of the Pharmacology–Biochemistry Branch, Biosciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas. Further reproduction is authorized to satisfy the needs of the U.S. Government. Animals involved in this study were maintained in accordance with the *Guide for Laboratory Animal Facilities and Care* as published by the National Academy of Sciences-National Research Council.

We previously described the effects of reduced pressure on hexobarbital sleeping time, metabolism, and brain levels in mice.[6] In this communication we are reporting the effects of reduced pressure on the metabolism *in vitro* of various substrates, as well as on the pharmacologic action of hexobarbital, pentobarbital and zoxazolamine. The data presented in this paper show that exposure to altitude will result in an alteration of the action and metabolism of some drugs, such as hexobarbital and zoxazolamine, but not of others, such as pentobarbital and phenobarbital. Our results also indicate that the change produced by altitude on drug metabolism *in vitro* is not always in the same direction.

Male C-57 black mice weighing 20–30 g were used for all experiments. Mice were exposed to altitude in a walk-in chamber divided into two compartments. One compartment of the chamber was evacuated to a pressure equivalent to 18,000 ft, while the other was maintained at ground level and used to house the control mice. All animals were kept in a wire cage in groups of 12–25 and allowed food and water *ad lib.* for the duration of the experiment.

The mice were kept at an altitude of 18,000 ft for 5 days, returned to ground level, and either sacrificed or injected with the drug under investigation after descent. In some experiments the mice were injected while still at altitude. Control mice were taken to 18,000 ft and then immediately returned to ground level in order to expose them to the stress of descent.

Metabolism *in vitro* of various drugs was measured using the method described by Kato and Gillette.[2] Each mouse liver was homogenized in 4 vol. of cold KCl solution (1·15%, w/v) in a glass homogenizer. The homogenate was centrifuged at 9000 *g* for 20 min at 4° in a Servall centrifuge. The final incubation mixture contained 3 ml of the 9000 *g* supernatant (equivalent to 0·6 g liver), 20 μmoles glucose 6-phosphate, 0·4 μmole NADP, 50 μmoles nicotinamide, 50 μmoles $MgCl_2$, 1·4 ml of 0·2 M phosphate buffer, pH 7·4, and one of the following substrates: 5 μmoles aminopyrine, aniline, methylaniline or *p*-nitrobenzoic acid; 4μmoles hexobarbital or phenylbutazone; 3 μmoles phenobarbital, *p*-nitroanisole or zoxazolamine; 2 μmoles pentobarbital. The total volume of the incubation mixture was 5 ml. All incubations were carried out at 37° in a Dubnoff metabolic shaker for 30 min under air, except for the mixtures containing *p*-nitrobenzoic acid which were incubated under nitrogen.

Alkyl hydroxylation of hexobarbital, pentobarbital, phenobarbital and phenylbutazone was measured by following the disappearance of substrate.[7–9] Aromatic hydroxylation of aniline was assayed by determining the formation of *p*-aminophenol,[10] and aromatic hydroxylation of zoxazolamine was estimated by the disappearance of substrate.[11] Demethylation of *N*-methylaniline was determined by measuring the amount of formaldehyde formed.[12] Demethylation of aminopyrine was estimated by measuring the formation of 4-amino-antipyrine.[13] The formation of *p*-aminobenzoic acid was used to measure the nitro-reduction of *p*-nitrobenzoic acid.[14] The activity of NADPH-dichlorophenol-indophenol reductase was determined by the method of Williams and Kamin.[15]

All drugs were dissolved in saline except for zoxazolamine, which was dissolved in a stoichiometric quantity of HCl, and injected intraperitoneally. The pharmacologic activity of hexobarbital and pentobarbital was measured by the duration of sleeping time (loss of righting reflex) and that of zoxazolamine by determining the duration of paralysis.

Student's *t*-test was used to assess the statistical significance of the results.

Sleeping time produced by hexobarbital (125 mg/kg) was of a shorter duration in the mice kept at altitude than in ground level controls (Table 1). This difference was observed whether they were injected after being returned to ground level or whether they were injected in the chamber while still at

TABLE 1. DRUG-INDUCED LOSS OF RIGHTING REFLEX IN MICE AT 18,000 ft AND AT GROUND LEVEL*

Drug	Dose (mg/kg)	Ground (min)	Altitude (min)	N	P
Hexobarbital	125	85·1 ± 5·8	55·1 ± 3·8	20	< 0·001
Zoxazolamine	90	52·2 ± 3·3	43·2 ± 2·8	15	< 0·05
Pentobarbital	40	29·2 ± 2·1	27·9 ± 2·6	20	NS
Pentobarbital	43	43·9 ± 4·1	49·0 ± 3·5	13	NS
Pentobarbital	50	83·9 ± 9·6	88·5 ± 6·7	11	NS
Hexobarbital	125†	78·7 ± 3·6	65·8 ± 5·2	20	< 0·05

*Mice were injected i.p. with the drug indicated. Each value is the mean ± S.E.M. of the duration of loss of righting reflex. N is the number of animals used in each group. NS = not significant.
†Altitude group injected while at 18,000 ft.

18,000 ft. No difference was apparent when pentobarbital (40 mg/kg) was used as the test drug. To determine if this lack of difference was due to the short duration of sleeping time at this dose, two higher doses of pentobarbital were used. Although the duration of sleeping time almost tripled at the higher dose (50 mg/kg), there was still no significant difference between the altitude and the ground control groups.

Administration of zoxazolamine (90 mg/kg) produced a significant decrease ($P < 0.05$) in the duration of paralysis between the altitude mice and the ground controls.

The rate of metabolism *in vitro* of several drugs was altered in the livers of altitude-exposed mice (Table 2). Increased metabolism of phenylbutazone, aniline, hexobarbital and zoxazolamine was observed in the altitude group. Conversely, demethylation of aminopyrine and the reduction of *p*-nitrobenzoic acid were slower in the altitude mice than in the ground level mice. There were no significant differences in the metabolism of *p*-nitroanisole, phenobarbital, methylaniline and pentobarbital. The activity of NADPH-dichlorophenol-indophenol reductase was also higher in the altitude mice than in the ground level controls. The greatest increase in metabolic activity of the altitude group occurred in the oxidation of phenylbutazone, while the greatest decrease was seen in the reduction of *p*-nitrobenzoic acid.

Our results show that the pharmacologic action of hexobarbital and zoxazolamine, but not that of pentobarbital, is decreased in mice kept at 18,000 ft for 5 days. A decreased hexobarbital sleeping time in mice maintained at 19,000 ft up to 14 days has previously been reported, although the decreased effect did not occur until after the second day at altitude.[16] On the other hand, others have reported an increased hexobarbital sleeping time at 18,000 ft, but these studies were performed using rats maintained at this altitude for only 35 min.[17]

Altitude has been shown to alter the pharmacologic action of several drugs. The decrease in motor activity in mice produced by chlorpromazine and meprobamate is potentiated at altitude.[18] An increase in the toxicity of reserpine, but not that of phenobarbital or tranylcypromine, as well as a decrease in semicarbazide-induced convulsions was observed in mice taken to 19,000 ft.[4, 19] A reduction in the toxicity of morphine at altitude was shown by Nedzel.[20]

Our experiments also show that there is an alteration in the metabolism *in vitro* of several drugs in livers from mice maintained at altitude, although the change was not consistently in the same direction. Thus the rate of metabolism was either increased, decreased or unchanged, depending on the substrate used. These varied rates of metabolism of different drugs produced by the stress of altitude are similar to results reported to occur with other forms of stress. Exposure to cold increases the metabolism of aniline and ethylmorphine in rats, but decreases the metabolism of hexobarbital.[21, 22] Starvation of mice decreases the metabolism of hexobarbital, chlorpromazine, aminopyrine and acetanilide, but not the reduction of *p*-nitrobenzoic acid and neoprontosil.[23] In rats starvation may increase or decrease drug metabolism, depending upon the sex of the animals.[2]

The increased rate of metabolism of phenylbutazone, aniline, hexobarbital and zoxazolamine may be due to an altitude-induced increase in activity or the quantity of the drug-oxidizing enzymes in the microsomes. An increase in the synthesis of tryptophan oxygenase and tyrosine-α-ketoglutaric acid is produced in mice kept 17 hr at altitudes ranging from 16,750 to 28,900 ft.[24] It was suggested that the induction of these enzymes was mediated by adrenocortical hormones. Others have also postulated that these hormones are involved in changes in the rate of metabolism and action of drugs produced by stress.[25] Increased enzyme synthesis produced by the stress of altitude would result in an enhanced rate of metabolism of hexobarbital and zoxazolamine in liver and could account, in part for the decreased pharmacologic activity of both drugs in the altitude mice. This suggestion is further supported by the fact that there was no increase in the metabolism of pentobarbital and also no difference in the sleeping time between the altitude and ground level mice injected with this drug. This explanation must assume that: (1) different enzymes are responsible for the metabolism of hexobarbital, zoxazolamine and pentobarbital; and (2) altitude does not affect these enzymes in the same way. We have previously demonstrated that these altitude conditions do not affect brain receptor sites; however, the possibility exists that an alteration in tissue distribution or rate of excretion of these drugs in the altitude mice may also be important factors in decreasing their pharmacologic action.

The emphasis in most experiments conducted to determine the effects of reduced pressure on experimental animals has been on the stress produced by the attendant hypoxia.[17, 24, 26] Our experimental conditions were such that the mice would experience a certain degree of hypoxia at 18,000 ft, since the oxygen tension would be approximately 50 per cent less than at sea level. However, there are several

TABLE 2. EFFECT OF ALTITUDE ON THE ACTIVITY OF MICROSOMAL ENZYMES IN MOUSE LIVER*

Substrate		Ground level (nmoles/g wet wt./hr)	Altitude (nmoles/g wet wt./hr)	N	Per cent of control	P
Oxidations						
Phenylbutazone	Aliphatic hydroxylation	433 ± 78	670 ± 43	6	+ 155	< 0·05
Phenobarbital	Aliphatic hydroxylation	1710 ± 126	2001 ± 75	12	+ 117	NS
Hexobarbital	Aliphatic hydroxylation	1706 ± 228	2618 ± 295	8	+ 153	< 0·05
Pentobarbital	Aliphatic hydroxylation	1087 ± 72	1018 ± 89	12	− 6	NS
Zoxazolamine	Aromatic hydroxylation	458 ± 17	529 ± 19	12	+ 115	< 0·02
Aniline	Aromatic hydroxylation	710 ± 41	938 ± 71	12	+ 132	< 0·02
Nitroanisole	*O*-demethylation	408 ± 25	489 ± 35	12	+ 119	NS
Aminopyrine	*N*-demethylation	732 ± 42	605 ± 40	12	− 17	< 0·05
Methylaniline	*N*-demethylation	676 ± 61	590 ± 45	11	− 13	NS
Reductions						
Nitrobenzoic acid		319 ± 15	251 ± 12	12	− 21	< 0·01
Dichlorophenol-indophenol		122,700 ± 6760	156,900 ± 7900	22	+ 128	< 0·01

*The activities are expressed as the means ± S.E.M. of the values obtained using a 9000 *g* supernatant fraction of liver and cofactors in amounts stated in the text. N is the number of animals used in each group. NS = not significant.

reports in which an alteration of drug action at altitude occurred in an environment containing ground equivalent oxygen.[20, 27, 29] Although our experiments do not separate the effect of hypoxia from that of reduced pressure, it is conceivable that both conditions may act in concert or individually to alter drug action and metabolism.

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