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Effect of Pentobarbital and Gaseous Anesthetics on Rats Selectively Bred for Ethanol Sensitivity

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DEITRICH, R. A., L. J. DRASKI AND R. C. BAKER. *Effect of pentobarbital and gaseous anesthetics on rats selectively bred for ethanol sensitivity.* PHARMACOL BIOCHEM BEHAV **47**(3) 721–725, 1994.—.Rats have been genetically selected to have a differential hypnotic response to an acute injection of ethanol. These high alcohol sensitive (HAS) and low alcohol sensitive (LAS) rats were used to investigate commonalities of the mechanism of action of several gaseous anesthetics, pentobarbital and ethanol. Similar studies have been carried out extensively with mouse lines also differentially sensitive to ethanol (short- and long-sleep mice). Like the mice, the rats are also differentially sensitive to the two gaseous anesthetics, enflurane and isoflurane. However, in contrast to results with these mice, we find that the HAS and LAS rats are differentially sensitive to halothane and pentobarbital in the same direction as their sensitivity to ethanol. In other studies, the rats also have been found to be differentially sensitive to phenobarbital as are SS and LS mice. These results show that, by the use of these anesthetics in combination with selectively bred rodent lines, many new opportunities for dissecting the molecular mechanisms of anesthetic agents present themselves.

Selective breeding HAS LAS Anesthetics Genetics Rats

A GREAT deal of information has been obtained by the use of rodents selectively bred for differential responses to ethanol (10). The process is such that by application of bidirectional artificial selection pressure for a particular behavioral response to ethanol for many generations, animals are produced that carry the genes for an increased or decreased response. With continued selection, the genes responsible for the selected phenotype become homozygous and, therefore, fixed in the different lines. All other genes, with the exception of those fixed by random chance, segregate within each line (7). Thus, for animals selectively bred for responses to ethanol, differences in responses to other anesthetic agents should not occur, unless those agents have a significant overlap in the mechanism of action with ethanol.

One of the most successful applications of these principals has been in the study of the GABA-chloride channel system (9,15,17,22,23,27–29). The basic conclusion from these studies is that a variety of anesthetic and central nervous system depressing agents have at least a portion of their mechanism of action due to the augmentation of the GABA-stimulated chloride flux into neurons. The list of these agents includes ethanol (14,17,26,35), barbiturates (17,37), benzodiazepines (32), gaseous anesthetics (22,27,39), some insecticides (28), propofol (3–5), and anesthetic steroids (6,16,18).

The GABA receptor system in the brain is made up of unique combinations of various subunits, with five subunits per receptor. The composition of these subunits, which make up individual receptor subtypes, dictates the characteristics of that receptor. Apparently there are receptor sites for benzodiazepines, barbiturates, and ethanol [see review (9)]. The distribution of the known receptor subunits has been mapped in rodent brain (20,21,38). Perhaps one of the best demonstrations of the specificity of the actions of these receptors is in the finding of Wafford et al. (36). In this study, one particular splice variant of the gamma 2 subunit is required for the receptor to be responsive to ethanol. This splice variant has eight

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amino acids more than its shorter form, with a consensus sequence for protein kinase C phosphorylation contained within these amino acids.

Given the success in dissecting the actions of ethanol and other anesthetic agents by using the SS and LS mice, we undertook to see if these observations extended to a similar selection, but in rats. The possibility that there would be subtle differences between the two selections with regard to GABA function provided the opportunity to analyze further the actions of anesthetic agents.

Originally, we had tested HAS and LAS rats for their sensitivity to pentobarbital in generation 8 of selection (11). There we found that the LAS rats were less sensitive to pentobarbital. In generation 18, we noticed some results that indicated that this might no longer be true. We, therefore, undertook a much more extensive investigation of the actions of pentobarbital than previously. In addition, we included a study of the actions of several gaseous anesthetics.

METHODS

Rats, selectively bred to be sensitive (HAS) or resistant (LAS) to acute doses of ethanol were used. Results from animals of generation 8 are included for comparison purposes but the animals used in the current experiment were from generations 18-20 (selected generations 17-19). Both male and female rats were used. Approximately equal numbers of each replicate were used (i.e., HAS_1 and HAS_2 replicates). The body mass of each group is given in the experiments with pentobarbital.

Pentobarbital Experiments

Rats were injected with doses of pentobarbital intraperitoneally. Rats obtained from generation 8 were given 60 mg/kg. However, this dose resulted in excessively long times of loss of the righting response for rats of generation 18-20. Consequently, the data reported here (generation 18-20), reflect doses of either 30 or 35 mg/kg. While times for loss of the righting response (the time from injection until the animals were able to right themselves in a V-shaped trough) were recorded, the more informative data are the brain levels at the time of regain of the righting reflex. Because there was no difference in the brain values following 30 or 35 mg/kg, these data have been combined for each line. At the regain of the righting reflex, the animals were decapitated and the brain homogenized in water to give a 10% homogenate. An aliquot of this homogenate was taken and pentobarbital determined by gas chromatography as outlined previously (11). Metabolic rates were calculated from these data by taking the amount of pentobarbital injected minus that found at regain of the righting response divided by the time elapsed. These values are reported as ug pentobarbital disappearing per minute per kg body weight.

Pentobarbital metabolism was determined using male rats. The rats were injected with 35 mg/kg pentobarbital. A retroorbital blood sample was obtained at 30-min intervals to 180 min. Blood pentobarbital was determined as for brain. The first order rate constants are calculated from the slopes of the lines resulting from plotting log of blood pentobarbital concentration vs. time. The half-life was then calculated from these values.

Gaseous Anesthetic Experiments

Gaseous anesthetics were given without dilution by injection with a microsyringe intraperitoneally. From pilot experiments, the doses needed to give approximately equivalent sleep times were determined. As is apparent, these doses were markedly different for each line. The sleep time was recorded and the brain was homogenized in water to give a 10% homogenate. The homogenate was frozen at $-70°$ until analyzed. A 0.5 ml aliquot of the homogenate and 1.5 ml H_2O containing the internal standard was heated to 60°C. A 1 ml sample of head space gas was removed and the amount of anesthetic determined by gas chromatography. The concentration of each anesthetic was determined based on standard curve prepared for each anesthetic analyzed.

Statistical Analysis

Student's t-test was used for all comparisons reported. Statistical significance was set at $p < 0.05$.

RESULTS

Sensitivity to Pentobarbital

Table 1 gives the results of these experiments. Originally in generation 8 we had found that older male rats of the LAS

TABLE 1

BRAIN LEVELS OF PENTOBARBITAL AT AWAKENING IN HAS AND LAS RATS

Line	Sex	Weight	Level	p	Metab.
HAS	F	111.1 ± 4.9 (15)	16.92 ± 0.9	<0.0001	72 ± 6
LAS	F	114.9 ± 5.3 (14)	22.57 ± 1.07		71 ± 5
HAS	F	220.8 ± 8.5 (9)	15.4 ± 1.46	0.0086	51 ± 4
LAS	F	200.1 ± 12.9 (10)	20.32 ± 0.9		44 ± 4
HAS	M	131.7 ± 6.3 (18)	15.92 ± 1.09	0.0047	168 ± 16
LAS	M	131.0 ± 6.9 (17)	19.9 ± 0.67		198 ± 24
HAS	M	343.0 ± 21.9 (10)	19.87 ± 1.33	NS	158 ± 17
LAS	M	341.6 ± 15.4 (7)	20.54 ± 1.71		165 ± 36
HAS*	M	362.9 ± 15.2 (11)	16.38 ± 1.05	0.0032	237 ± 12
LAS*	M	347.5 ± 10.2 (12)	20.76 ± 0.79		324 ± 22

Weight is in grams. Brain levels are μg/g of brain. Data for all experiments are included regardless of dose or generation. Metabolism is μg/kg/min (calculated from the injection dose and brain levels at time of awakening).

*Data from Draski et al. (11). Rats were given 60 mg/kg in that experiment.

TABLE 2

RATES OF DISAPPEARANCE OF PENTOBARBITAL FROM MALE HAS AND LAS RATS

Line (*n*)	Weight (g)	Half Life (min)
HAS (10)	290 ± 9.4	55.4 ± 8.4
LAS (9)	255 ± 7.3	54.6 ± 10.3

line were significantly less sensitive to pentobarbital than male rats of the HAS line, as shown by the fact that the LAS awoke at a higher brain pentobarbital levels. A 60 mg/kg dose was used in the previous experiment and the times of absence of the righting response were quite long (>180 min). When we attempted to replicate these results using lower doses of pentobarbital, we found in the first experiment that there was now no significant difference in the brain levels of pentobarbital at awakening. We then carried out more extensive studies of both male and female rats. In all these subsequent experiments we found that the LAS rats were consistently less sensitive to pentobarbital than were the HAS rats. In addition, female rats had considerably longer times for loss of the righting response than their male counterparts. The difference between female and male time of loss of the righting response was due to differences in metabolic rates. The rate of pentobarbital metabolism was about threefold higher in male rats as compared to female rats. This calculation is based on the time elapsed and concentration of pentobarbital at the time the rats regained the righting reflex. One must assume an equal distribution of pentobarbital in the body, and the dose of pentobarbital injected is taken as the body level at time zero. While this method provides an estimate of pentobarbital metabolism, it is not as precise as direct studies of the rates of disappearance of pentobarbital. The results of these studies confirm that the difference between HAS and LAS rats is not metabolic in nature (Table 2). Quinn et al. (30) also found a marked sex difference for the metabolism of butabarbital for female and male rats that were 5 weeks old or older.

As is clear from Table 3, LAS rats were much less sensitive to the anesthetic effects of the gaseous anesthetics halothane, enflurane, and isoflurane. The brain levels at regaining the righting response in LAS were close to double that for HAS for all three anesthetics.

For comparison purposes, the blood levels of ethanol at regain of the righting reflex at generations 18, 19, and 20 are given in Table 4.

TABLE 4

ETHANOL BLOOD LEVELS (mM) AT REGAIN OF RIGHTING REFLEX FOR MALE HAS AND LAS RATS

Line	Generation	BAC (mM) ± SEM
HAS	18	63 ± 1.5
LAS	18	105 ± 1.0
HAS	19	48 ± 1.2
LAS	19	86 ± 1.0
HAS	20	55 ± 1.4
LAS	20	99 ± 1.0

DISCUSSION

It is clear that the selection for initial ethanol sensitivity in rats produces phenotypes very similar to those resulting from the same selection in mice (8,10,11). However, there are several important exceptions that may provide clues, not only to the differences in selection, but to the mechanism of action of other anesthetic agents. While there has been some debate as to the actions of pentobarbital in the SS and LS mice, it is established that the differences found are due to pharmacokinetic and not to pharmacodynamic differences between the SS and LS mice (12,13,31,33). An extensive study of many hypnotic agents in SS and LS mice has been carried out by Marley et al. (25). Because it also is well established that there are differences in the GABA receptor chloride channel system between SS and LS mice, one must assume that this does not extend to the actions of pentobarbital on this receptor. On the other hand, just the opposite situation exists for the HAS and LAS rats. In this case, there is also convincing evidence that the GABA receptor chloride channel system is differentially sensitive to ethanol, benzodiazepines, phenobarbital, and pentobarbital. Allan et al. (1) found that microsacs from the brains of HAS rats were more sensitive than those from LAS rats to the effects of pentobarbital, phenobarbital, flunitrazepam, and ethanol on GABA-mediated chloride flux. This agrees well with the behavioral data. The simplest assumption is that the difference between the receptor system in HAS and LAS rats is responsible for the variations in response. Of course, it is possible that the differences lie in specific combination of receptor subunits and were simultaneously selected by the breeding process. Uusi-oukari and Korpi (34) have studied the GABA receptor system in the AT (alcohol tolerant) and ANT (alcohol nontolerant) rats that were selec-

TABLE 3

BRAIN LEVELS OF ANESTHETICS AT AWAKENING IN HAS AND LAS RATS

Compound	Line	Dose	Levels	*p*	Sleep Time	*p*
Halothane	HAS	0.5	35.28 ± 4.58 (10)	0.0269	37.5 ± 3.9	NS
Halothane	LAS	1.0	67.58 ± 13.1 (9)		40.8 ± 5.5	
Isoflurane	HAS	1.4	21.39 ± 3.21 (8)	0.0011	81.0 ± 14.5	NS
Isoflurane	LAS	2.3	50.44 ± 6.01 (10)		65.9 ± 9.0	
Enflurane	HAS	2.0	42.12 ± 6.52 (10)	0.0094	85.9 ± 5.7	0.0035
Enflurane	LAS	3.36	75.12 ± 8.95 (12)		59.9 ± 5.6	

Dose is in g/kg. Levels are in μg/g brain. Time is in minutes. All ± SEM.

tively bred for differential sensitivity to the ataxic effects of ethanol. They found an enhanced interaction between GABA and a benzodiazepine agonist and suggested that altered binding sites could be responsible for the increased sensitivity of the ANT rats to hypnotics.

The rats have been selected for loss of righting response caused by ethanol, but they also show a number of other differences on various behavioral measures including ethanol-induced hypothermia, ataxia, acute tolerance, as well as thyrotropin releasing hormone reversal of ethanol induced ataxia and benzodiazepine sensitivity [see (9) for a review].

There was a large difference in the time of loss of the righting response produced by a given dose of pentobarbital in male vs. female rats of either the HAS or LAS lines. However, the brain levels upon awakening were the same within a given line regardless of the sex. Studies of the metabolic rates as shown in Table 1 confirm that there is a large difference in the metabolism of pentobarbital between male and female rats. However, this table, as well as Table 2, show that there is no difference between the lines in the metabolic rate. There is reasonable agreement between the data for the males in Tables 1 and 2. The average rate of disappearance for males in Table 1 is 172 μg/min/kg or 10 mg/kg/h for those animals given 30 or 35 mg/kg. The half-life of 55 min would predict that following this dose, the rate of metabolism should be 17.7 mg/kg/h. Previously we have found that male HAS and LAS rats did not differ in the activity of various cytochrome P450 enzymes (1A1, 1A2, 2B1, 2E1) (24), consistent with the observed equal metabolic rates between HAS and LAS male rats observed in these experiments.

In the case of the gaseous anesthetics, earlier reports had found that there was no difference in the dose-response curve for halothane between the LS and SS mice (2). Others reported a small difference in the lines to the anesthetics (19). In this case, the measure was the percentage of anesthetic in a closed chamber which was necessary to cause loss of the righting reflex. In neither of these cases were brain levels of the anesthetic measured. The current report clearly shows that there is a nearly twofold difference in the sensitivity of the HAS vs. the LAS lines of rats to all three anesthetic agents. A recent study has confirmed that there is no difference in the sensitivity of SS and LS mice to halothane, but there is a difference in sensitivity to isoflurane (Baker et al., in preparation). In this case, brain levels at the regain of the righting response were measured. Taken together, these results show that the mechanism of action of halothane in mice is clearly different from that of the other anesthetics. However, the selection for ethanol sensitivity in the rats did not result in such a difference. In the face of these data it is difficult to maintain that the action of gaseous anesthetics is based solely on bulk membrane effects. Halothane is a halogenated hydrocarbon, while the other agents are halogenated ethers. Whether or not this is significant in the studies with mice remains to be seen. In any case, the selection for ethanol sensitivity clearly has resulted in a similar selection for sensitivity to lipid soluble gaseous anesthetics and to pentobarbital in rats.

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