*Pharmacology Biochemistry & Behavior*, Vol. 16, pp. 145–149, 1982. Printed in the U.S.A.

# Regional Brain Levels of Monoamines in Alcohol-Preferring and -Nonpreferring Lines of Rats[1]

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Received 6 July 1981

MURPHY, J. M., W. J. McBRIDE, L. LUMENG AND T.-K. LI. *Regional brain levels of monoamines in alcohol-preferring and -nonpreferring lines of rats.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 145–149, 1982.—Regional brain levels of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA) and norepinephrine (NE) were determined in alcohol-naive rats from lines selectively bred for alcohol preference (P) and alcohol aversion (nonpreference, NP). Based on comparison by a standard *t*-test, the P rats had 12% lower NE in the pons-medulla, 20% higher NE and 16% lower DA content in the cerebral cortex (CX) than did the NP rats. However, the predominant finding was that the levels of 5-HT and 5-HIAA were 12–26% lower for the P than for the NP rats in the CX, hippocampus (HIP), corpus striatum (STR), thalamus (TH) and hypothalamus (HY). Regional CNS monoamines from a group of independently bred, stock Wistar rats were also compared with the NP and P groups to determine if the selectively bred rats differed widely from an unselected population. In most instances, the NP and P rats fell within the range of the stock group. When the stock group was included in an analysis of variance of the data, post-hoc differences between the NP and P groups that remained significant were the lower levels of 5-HT (and in some cases 5-HIAA) in the CX, HIP, STR, TH and HY of the P group. In the HY and HIP, the 5-HT levels of the P and NP animals diverged significantly in opposite directions from those of the stock group, possibly suggesting an involvement of these regional serotonergic systems in alcohol preference.

Alcohol-preferring rats  Regional brain monoamines  Norepinephrine  Dopamine  Serotonin  5-Hydroxyindoleacetic acid

SEVERAL neurotransmitters have been implicated as mediators of the effects of ethanol and alcohol-drinking behavior, and norepinephrine (NE), dopamine (DA) and serotonin (5-HT) have received considerable attention in this regard. Some experimental findings suggest that various aspects of these neurotransmitter systems may be related to alcohol sensitivity, tolerance and addiction [9, 15, 22]. To date, however, a clear role for any neurotransmitter in the mediation of alcohol-drinking behavior has yet to emerge.

Inbred and selectively bred lines of animals exhibiting alcohol preference and aversion (nonpreference) have been employed to discern the neurochemical correlates of alcohol-drinking behavior [7,9]. In our laboratory, two rat lines of Wistar origin have been selectively bred for their alcohol-preferring (P) and alcohol-nonpreferring (NP) drinking behavior [14, 17, 18]. The P-line of rats, in a free-choice situation with 10% ethanol, water and food available ad lib, voluntarily consumes amounts of ethanol (> 5 g/kg body weight/day) approaching their metabolic capacity, whereas rats of the NP line almost totally avoid the 10% alcohol solution [13,14].

In an earlier, exploratory study on the behavioral and neurochemical characteristics of the NP and P lines, some differences in brain monoamine content were observed [20]. However, because there was still overlap in the alcohol consumption scores of the P and NP rats in the S8 generation, the P and NP groups of rats in that study were first tested for alcohol preference before neurochemical analysis. Research on two other rat lines selectively bred for alcohol preference and nonpreference, the Alko alcohol (AA) and Alko nonalcohol (ANA) lines, has also yielded some differences in brain monoamine content [1, 2, 3, 4], whereas studies on the in-

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TABLE 1

NOREPINEPHRINE LEVELS IN SEVEN BRAIN REGIONS OF NONPREFERRING (NP), PREFERRING (P) AND STOCK WISTAR RATS

| | nmoles/g wet wt. (mean ± S.E.M.) | | |
|---|---|---|---|
| | NP (N=12) | P (N=12) | Stock (N=15) |
| Cerebral Cortex | 1.28±0.07† | 1.54±0.05* | 1.76±0.13 |
| Corpus Striatum | 1.17±0.11 | 0.97±0.12 | 1.18±0.21 |
| Thalamus | 3.08±0.12 | 3.46±0.27 | 3.40±0.33 |
| Hypothalamus | 8.22±0.37† | 8.09±0.50 | 10.14±0.81 |
| Hippocampus | 1.04±0.08 | 1.24±0.13 | 1.22±0.18 |
| Midbrain | 2.10±0.12† | 2.13±0.10† | 2.63±0.18 |
| Pons-Medulla | 3.58±0.07 | 3.14±0.12* | 3.74±0.25 |

*$p<0.05$ for P compared to NP group ($t$-test).
†$p<0.05$ with respect to values for stock group (Newman-Keuls test).

TABLE 2

DOPAMINE LEVELS IN SEVEN BRAIN REGIONS OF NONPREFERRING (NP), PREFERRING (P), AND STOCK WISTAR RATS

| | nmoles/g wet wt. (mean ± S.E.M.) | | |
|---|---|---|---|
| | NP (N=12) | P (N=12) | Stock (N=15) |
| Cerebral Cortex | 7.34±0.36 | 6.17±0.28* | 6.40±0.39 |
| Corpus Striatum | 62.72±2.20 | 62.85±2.50 | 58.63±3.92 |
| Thalamus | 2.32±0.24 | 3.61±0.80 | 4.23±0.73 |
| Hypothalamus | 2.27±0.42 | 2.14±0.14 | 2.85±0.39 |
| Hippocampus | 0.28±0.04 | 0.31±0.04 | 0.41±0.09 |
| Midbrain | 1.16±0.12 | 1.08±0.10 | 1.33±0.14 |
| Pons-Medulla | 0.34±0.02 | 0.30±0.01† | 0.38±0.02 |

*$p<0.05$ for P compared to NP group ($t$-test).
†$p<0.05$ with respect to values for stock group (Newman-Keuls test).

bred, alcohol-preferring (C57BL) and alcohol-avoiding (DBA) strains of mice have revealed no differences in the whole brain content of 5-HT, NE or DA [8,21]. These studies have not shown consistent differences in monoamine levels between the animals with opposing alcohol-drinking behavior, but comparisons may be misleading owing to differences in methodology and experimental design. For example, in some of the studies, the animals had been exposed to alcohol for varying periods of time; thus the effects of previous exposure to alcohol cannot be separated from innate differences (e.g., [20]). Other studies assayed only whole brain levels, thereby masking potentially important regional differences.

The objective of the present study was to determine innate differences between the P and NP lines of rats in the CNS content of NE, DA, 5-HT and the primary metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA). Importantly, it was designed to avoid some of the potentially confounding factors present in previous studies by (a) using animals that had never been exposed to alcohol; (b) studying regions within the CNS instead of whole brain; and (c) killing the animal and dissecting the CNS regions under conditions that minimize postmortem changes. For comparison, a group of stock Wistar rats was similarly studied to determine if there were any consistent differences that would distinguish the NP or P lines from an independently bred, heterogeneous population, and to ensure the adequacy of the assay procedure in the event that both the NP and P groups showed some neurochemical anomaly.

## METHOD

The selectively bred P and NP lines originated from a randomly bred, Wistar (Wrm:WRC(WI)BR) colony at the Walter Reed Army Institute of Research [17]. The methods employed to develop these lines have been described [14]. The present study utilized male NP- (N=12) and P-line (N=12) rats from the S18 generation and a group (N=15) of randomly bred, male Wistar rats (Harlan Industries, Indianapolis, IN). All rats were housed individually and were never exposed to alcohol. Food (Wayne Lab Blox, Allied Mills) and water were provided ad lib and normal 12 hr light-dark cycles were maintained beginning at 0800 hr. The animals were handled daily for at least seven days prior to liquid $N_2$ dipping and were adapted to the killing apparatus in order to minimize stress.

At approximately 90 days of age, the rats were killed within the same time period each day (1300–1500 hr) by the near-freezing method [25]. The brains were rapidly removed and dissected at −20°C or lower into seven areas: cerebral cortex (CX), hippocampus (HIP), hypothalamus (HY), midbrain (MID), pons-medulla (PM), corpus striatum (STR) and thalamus (TH). The olfactory bulbs and cerebellum were discarded. The brain parts were stored at −70°C until assayed for the content of NE, DA, 5-HT and 5-HIAA by HPLC with electrochemical detection [16]. The compounds were extracted from the frozen tissue by homogenization in at least 15 volumes of ice-cold 1 N formic acid/acetone (15/85:v/v). Tubes containing the homogenates were allowed to sit on ice for 45 min with periodic vortexing, and then were centrifuged for 10 min at 900×G. Aliquots of the clear supernatants were placed in separate tubes and washed with two volumes of heptane/chloroform (8/1:v/v). The samples were centrifuged for 5 min at 300×G to separate phases, and the organic layer was aspirated and discarded. The aqueous phase was evaporated in a vacuum centrifuge. The dry residues containing the compounds of interest were resuspended in 1 ml of the HPLC buffer, and 200 μl of each sample was injected on the HPLC. The aqueous HPLC mobile phase (pH 3.5) contained 0.069 M citric acid, 0.035 M disodium phosphate, 0.35 mM octyl sodium sulfate, 0.10 mM EDTA, and 20% methanol (v/v) was added. Flow rate was maintained at 1.0 ml/min through a C-18 reverse-phase column. A glassy carbon detector was set at 0.8 volts vs a Ag/AgCl reference electrode, and the amperometric controller was set at 10 nA/volt. Peak areas were determined by integration and compound contents were quantitated from standard curves. Dihydroxybenzylamine was added as an internal standard during the extraction procedure, and recovery of authentic standards for the extraction procedure generally ranged from 90–95%. The precision of the method was ±2–4% (CV). Each of the assays was designed to detect recovered compounds in concentrations of at least 2 pmole per 200 μl injection.

Statistical comparisons between the selectively bred NP and P groups were made by a standard $t$-test for independent groups. In addition, the NP, P and randomly bred stock Wis-

TABLE 3

SEROTONIN LEVELS IN SEVEN BRAIN REGIONS OF NONPREFERRING (NP), PREFERRING (P) AND STOCK WISTAR RATS

| | nmoles/g wet wt. (mean ± S.E.M.) | | |
|---|---|---|---|
| | NP (N=12) | P (N=12) | Stock (N=15) |
| Cerebral Cortex | 3.95±0.14† | 3.32±0.08*‡ | 3.25±0.13 |
| Corpus Striatum | 2.63±0.12 | 2.21±0.11*‡ | 2.18±0.19 |
| Thalamus | 5.18±0.10† | 4.46±0.14*‡ | 4.37±0.19 |
| Hypothalamus | 4.68±0.24 | 3.71±0.24*‡ | 4.46±0.28 |
| Hippocampus | 2.73±0.08 | 2.13±0.07*‡ | 2.44±0.16 |
| Midbrain | 10.25±1.61 | 10.03±1.46 | 7.83±1.27 |
| Pons-Medulla | 4.33±0.14 | 4.44±0.17 | 4.56±0.25 |

*$p<0.05$ for P compared to NP group ($t$-test).
†$p<0.05$ with respect to values for stock group (Newman-Keuls test).
‡$p<0.05$ for P compared to NP group (Newman-Keuls test).

TABLE 4

5-HYDROXYINDOLEACETIC ACID LEVELS IN SEVEN BRAIN REGIONS OF NONPREFERRING (NP), PREFERRING (P) AND STOCK WISTAR RATS

| | nmoles/g wet wt. (mean ± S.E.M.) | | |
|---|---|---|---|
| | NP (N=12) | P (N=12) | Stock (N=15) |
| Cerebral Cortex | 1.93±0.06† | 1.62±0.05*‡ | 1.59±0.08 |
| Corpus Striatum | 1.97±0.16 | 1.67±0.10 | 1.67±0.16 |
| Thalamus | 3.18±0.12 | 2.81±0.11* | 2.88±0.23 |
| Hypothalamus | 1.60±0.13 | 1.22±0.11* | 1.54±0.12 |
| Hippocampus | 1.87±0.05 | 1.39±0.06*‡ | 1.67±0.15 |
| Midbrain | 3.49±0.12 | 3.71±0.20 | 3.71±0.35 |
| Pons-Medulla | 2.59±0.09 | 2.75±0.14 | 2.70±0.21 |

*$p<0.05$ for P compared to NP group ($t$-test).
†$p<0.05$ with respect to values for stock group (Newman-Keuls test).
‡$p<0.05$ for P compared to NP group (Newman-Keuls test).

tar groups were compared by analyses of variance and Newman-Keuls tests.

## RESULTS

No significant differences in body weights or CNS tissue weights were observed among the P, NP and stock Wistar groups (data not shown). The NE, DA, 5-HT and 5-HIAA content of CX, STR, TH, HY, HIP, MID and PM, of the three groups of rats are shown in Tables 1–4.

Because the stock Wistar rats were independently bred and not a concurrent, randomly bred line from the colony of origin for the NP and P lines, the NP and P groups were first compared separately by the $t$-test, as one approach to data analysis. Several significant differences were noted in comparison of the NP and P groups. In the P group, NE levels were 20% higher in the CX and 12% lower in the PM (Table 1) and DA content was 16% lower in the CX (Table 2) than in the respective brain parts of the NP group. In the CX, STR, TH, HY and HIP, the levels of 5-HT were 14–22% lower in the P than in the NP group (Table 3). The differences in 5-HIAA levels paralleled the differences in 5-HT (Table 4), except for the STR, where a statistically significant difference for 5-HIAA between the NP and P groups was not found.

If it is assumed that the independently bred, stock Wistar animals represent a valid control population for the NP and P lines, then the three groups can be compared by analysis of variance and significant differences among groups can be determined by the post-hoc Newman-Keuls test. Analyzed in this manner, the differences between the P and NP groups obtained by $t$-test for 5-HT remained significant, but the differences for the catecholamines did not. For 5-HIAA, the differences seen in the TH and HY by $t$-test were now not significant, but the differences in the CX and HIP remained. Comparison of the stock animals with the P and NP groups revealed that the stock Wistar group had higher NE levels in the CX, HY and MID than the NP group and greater NE content in the MID than the P group (Table 1). Serotonin levels in the CX and TH were lower in the Wistar animals than in the NP group (Table 3) and 5-HIAA levels in the CX showed a similar difference (Table 4). The only difference for DA occurred in the PM where higher DA content was found in the stock animals than in the P animals (Table 2).

## DISCUSSION

The use of the selective breeding of experimental animals as a pharmacogenetic approach to the study of alcohol-related behaviors has been recently reviewed [7,9]. The AA and ANA and the P and NP rats are lines that have been selectively bred for divergent voluntary ethanol-drinking behaviors [14,17]. Although the behavioral responses to ethanol administration have been compared in these rat lines in several studies [18, 19, 20], investigations of the neurochemical correlates of voluntary drinking behavior have thus far been limited. The present study represents the first neurochemical determinations in P and NP animals which had never been exposed to ethanol.

Ideally, in selective breeding experiments, a randomly bred, control line is maintained together with the selected lines to monitor, among other variables, random drift. Unfortunately, such a concurrent control line was not established either with the P and NP lines or with the AA and ANA lines at the outset of selective breeding. In this study, as a next-best measure, a group of independently and randomly bred Wistar rats were treated identically with the P and NP groups to ascertain whether selective breeding might have resulted in animals that differed widely from unselected Wistar rats. It was found that in most instances, the regional brain levels of NE, DA, 5-HT and 5-HIAA for both the P and NP groups fell within the range of values obtained for the stock animals. Where significant differences did occur, the P group more closely resembled the Wistar stock animals (two significant differences) than did the NP group (six significant differences). Only in the MID did both the P and NP groups differ from the stock group, with both selected lines exhibiting lower levels of NE than in the stock animals (Table 1). This isolated finding may have resulted from random drift, although a definitive conclusion can derive only from comparisons with a concurrent control line from the same base population.

The analysis and interpretation of the findings obviously depend upon whether or not the independently and randomly bred Wistar stock rats are considered an appropriate control group and are included in the analysis. When the stock group is not included in the analysis and the NP and P groups are compared by the *t*-test, some differences in brain catecholamine content were observed. Higher levels of NE were found in the CX of the P group than in the CX of the NP animals (Table 1). This finding is in agreement with the 20% greater NE content in the telencephalon of P rats reported previously [20] but contrasts with the report of a lower content of NE in the cerebral hemispheres of the AA than in the ANA animals [1]. Moreover, higher levels of DA were reported in whole brain [1,4] and in the limbic and striatal regions [4] of AA rats, whereas the present study found lower levels of DA in the CX of the P than of the NP group (Table 2).

The differences seen in this study between the NP and P groups for the catecholamines are consistent with findings from other studies suggesting that catecholamines may play an important role in certain responses to ethanol exhibited by animals with alcohol preference and nonpreference. For example, recent evidence suggests that the functional capacity of ascending forebrain DA systems may be directly related to alcohol sensitivity [12]. In the present study, lower DA content was found in the CX of the P-line animals, and it has been observed that P and AA rats are less sensitive to the depressant actions of ethanol than the NP and ANA rats, respectively [7, 14, 19]. The higher cortical NE content found in the P than in the NP rats is in agreement with studies suggesting that NE systems mediate the reinforcing properties of alcohol [5,6]. However, when the stock Wistar animals are included in the analysis of data as a control group, the regional brain catecholamine contents of the P and NP groups were not significantly different at the 0.05 level.

Whether or not the stock group is included in the analysis of the data, the P rats exhibited lower 5-HT levels in the telencephalic (CX, STR, HIP) and diencephalic (TH, HY) brain areas than the NP group (Table 3). The results for 5-HIAA tended to parallel the results for 5-HT (Table 4). These findings are, therefore, quite robust, suggesting that P-line rats have a lower metabolic rate, functional activity, and/or density of serotonergic pathways in the telencephalon and diencephalon than do the NP rats. This pattern does not agree with previous observations of no significant differences in the levels of 5-HT in the telencephalon or diencephalon-mesencephalon of P and NP animals (S8 generation), maintained on water only [20]. However, that study employed animals which had had free-choice access to ethanol for three weeks and then water only for one week prior to the time of killing. Therefore, those results may have reflected the CNS effects of alcohol consumption and/or withdrawal. Similarly, levels of 5-HT have been reported to be elevated in several brain regions of AA rats relative to values for ANA animals after four weeks of free-choice exposure to 10% ethanol and water [2,3].

Because the Wistar animals are heterogeneous in their voluntary ethanol-drinking behavior [14], one comparison that might reveal important differences may be the regional brain transmitter steady state levels of NP and P rats that change in directions opposite from those of stock animals as baseline (i.e., where the value for stock animals falls between significantly different values for the NP and P animals). This was the case for the steady state levels of 5-HT in the HIP and HY. Thus, at least one of these regional CNS differences could be indicative of a neurochemical continuum for alcohol preference and nonpreference.

The significance of the regional serotonergic differences observed between the NP and P groups is still difficult to evaluate owing to the currently equivocal state of the literature. Although the weight of evidence seems to favor no direct relationship between brain 5-HT systems and alcohol-drinking behavior [11,21], it cannot be ruled out that the 5-HT systems may interact with others [10,23] or that only specific regional aspects mediate alcohol preference, as exemplified by the differences in the HY and HIP seen here. The 5-HT systems are also thought by some investigators to play a role in the development of alcohol tolerance and dependence [23,24].

In general, the findings of this study do not agree with previous observations for the AA and ANA rats. The discrepancies may be attributable to some methodological differences such as the method of killing (near-freezing in liquid $N_2$ vs decapitation at room temperature) and dissection (at or below −20°C vs on ice). Moreover, it is well known that the CNS monoamine systems are sensitive to a number of behavioral and physiological variables which may have been different in different experiments. On the other hand, the fact that the AA and ANA lines were derived from a different stock population than the P and NP lines may also be contributory to the disparity. If the discrepancies in results are not due to methodologic variables, then the findings can be interpreted to suggest that these monoaminergic systems do not play a major role in the mediation of alcohol preference, since the AA and ANA lines also exhibit divergent voluntary alcohol-drinking behaviors. Support for this conclusion, however, requires additional experimental evidence to characterize other aspects of neuronal functioning, such as release, turnover and receptor interactions, that could be important CNS substrates for alcohol preference but are not detected by measuring steady state levels of neurotransmitters.

ACKNOWLEDGEMENTS

The authors would like to thank P. Hall and S. Cunningham for their excellent technical assistance.

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