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Alterations in Trace Amine and Trace Acid Concentrations in Isolated Aggressive Mice

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DOURISH, C. T., B. A. DAVIS, L. E. DYCK, R. S. G. JONES AND A. A. BOULTON. *Alterations in trace amine and trace acid concentrations in isolated aggressive mice.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1291–1294, 1982.—We have used an animal model of aggression, the isolation syndrome in mice, to examine the possible role of the trace amines, β-phenylethylamine (PEA), *meta*-tyramine (*m*-TA) and *para*-tyramine (*p*-TA) in aggressive behaviour. The brain, plasma and urinary levels of PEA, *m*-TA and *p*-TA, and their respective major acid metabolites, phenylacetic acid (PAA), *meta*-hydroxyphenylacetic acid (*m*-HPA) and *para*-hydroxyphenylacetic acid (*p*-HPA) were measured in isolated aggressive mice (after fighting), and in group housed controls. The urinary levels of PEA, *m*-TA, PAA, *m*-HPA and *p*-HPA, and the plasma levels of PAA and *p*-HPA were significantly lower in isolated aggressive mice. Similarly, the whole brain levels of PEA, *p*-TA, PAA and *p*-HPA tended to be reduced. In contrast, the brain levels of *m*-TA and *m*-HPA tended to increase. It should be noted, however, that the present procedure did not dissociate the stress and aggression components of the isolation syndrome, and, therefore, further experiments are required to determine whether the observed neurochemical changes are functionally related to increased aggression.

Aggressive behaviour Isolation syndrome Phenylethylamine Tyramine Stress

PROLONGED social isolation in male mice induces persistent and compulsive aggressive behaviour which is strain and sex dependent [17,20]. This experimental isolation syndrome [18] has been widely used as a tool to study the neurochemical and anatomical substrates of aggressive behaviour.

Isolation has been claimed to have no effect on whole brain 5-hydroxytryptamine (5-HT) levels, but to decrease 5-HT turnover particularly in the hypothalamus and midbrain [19,21]. In addition, a decrease in γ-aminobutyric acid concentration, a slight decrease in noradrenaline turnover, and an increase in dopamine turnover have been reported [14, 19–22]. However, the relationship between isolation-induced aggression and brain monoamine concentrations and turnover remains uncertain.

Recently, Sandler and colleagues [13] have claimed that phenylacetic acid (PAA), the major metabolite of the trace amine, β-phenylethylamine (PEA), is present in abnormally high levels in the plasma of aggressive psychopaths compared to non-violent control prisoners. On this basis, Sandler *et al.* [13] have proposed that PEA may be overproduced in violent offenders. In order to examine further the possible role of PEA and related trace amines, *para*-tyramine (*p*-TA) and *meta*-tryamine (*m*-TA), in aggressive behaviour, we have investigated the effects of prolonged social isolation in mice on the brain, plasma and urinary concentrations of PEA, *p*-TA and *m*-TA, and their principal acid metabolites PAA, *meta*-hydroxyphenylacetic acid (*m*-HPA) and *para*-hydroxyphenylacetic acid (*p*-HPA).

METHOD

Animals

The subjects were 60 male Swiss albino mice (Animal Resources, University of Saskatchewan) weighing 18–25 g at the beginning of the experiment. The mice were either housed individually (isolation) in metal cages (30×20×12 cm) or in groups of 10 in hanging wire cages (25×20×18 cm) for four weeks. Room temperature was maintained at 70–72°F and lighting operated on a 12 hr dark/light cycle (lights on 6 a.m.). Food and water were available ad lib.

Apparatus and Procedure

Testing was conducted in circular plastic metabolic cages (22×10 cm) designed for urine collection. Groups of 3 isolated or group housed animals were transferred to test cages where their aggressive behaviour was observed and rated on a standard scale [16] during alternate 5 min periods, for 30 min. A total of 10 groups of isolated mice and 10 groups of control mice were tested. Each group of mice was kept in a test cage for 24 hrs in order to collect urine samples.

Biochemical Determinations

At the end of the 24 hr test period the mice were subjected to ether anaesthesia and blood samples (0.5 ml) were collected from the heart into heparinized tubes. The tubes were centrifuged at 200 g for 10 min and subsequently at 2500 g for a further 10 min and the plasma removed. The animals were killed by decapitation and the brain minus the cerebellum and hindbrain was rapidly removed and frozen on dry ice. The 24 hr urine samples were diluted to 20 ml and 50 μl of concentrated HCl added as a preservative. Brain and plasma samples from individual animals in each group were pooled and stored at −70°F until analysed.

The brain, plasma and urinary levels of unconjugated

TABLE 1

CONCENTRATIONS OF UNCONJUGATED TRACE AMINES AND TRACE ACIDS IN THE BRAIN, PLASMA AND URINE OF ISOLATED AND GROUPED MICE

	Grouped Mice			Isolated Mice		
	Brain	Plasma	Urine	Brain	Plasma	Urine
PEA	1.5 ± 0.5 (7)		30.6 ± 2.6 (10)	1.4 ± 0.3 (8)		14.7 ± 1.7 (8)†
PAA	21.9 ± 3.0 (8)	74.9 ± 9.7 (10)	2860 ± 199 (10)	15.6 ± 2.2 (8)	40.0 ± 4.4 (10)*	1720 ± 240 (7)*
m-TA	2.0 ± 0.3 (7)		113 ± 8.9 (10)	3.1 ± 0.5 (8)		71.8 ± 8.6 (9)*
m-HPA	2.4 ± 0.2 (8)	4.2 ± 0.6 (10)	512 ± 48.0 (10)	3.0 ± 0.5 (8)	6.0 ± 2.4 (10)	227 ± 28 (9)†
p-TA	5.4 ± 0.6 (6)		103 ± 15.0 (7)	4.7 ± 0.5 (8)		87.3 ± 11.5 (8)
p-HPA	31.3 ± 2.8 (8)	108 ± 12.1 (10)	6760 ± 537 (10)	27.8 ± 1.9 (8)	64.2 ± 5.8 (10)*	4010 ± 516 (9)*

Trace amine and acid levels in brain are expressed as ng/g tissue, levels in plasma and urine are ng/ml.

All results are mean±SE with number of determinations in brackets. Symbols refer to statistically significant differences between isolated and grouped mice determined by a 2-tailed *t*-test for independent means: *$p<0.01$; †$p<0.001$. Trace amine concentrations in mouse plasma were below the limits of detection.

PEA, *p*-TA, *m*-TA, PAA, *m*-HPA and *p*-HPA were determined by direct probe mass spectrometric and gas chromatographic-mass spectrometric methods as previously described [4–8, 11, 12, 15].

RESULTS

Isolated mice were aggressive and showed significantly higher levels of fighting than group-housed controls (mean score during 30 min for isolated mice = 46.8, mean score during 30 min for grouped mice = 0.6; $p<0.01$, 1-tailed Mann Whitney U test). Isolated animals attacked other members of their group within 5 min of introduction to the test cage and continued to fight throughout the 30 min test.

The concentrations of unconjugated trace amines and unconjugated trace acids in the brain, plasma and urine of isolated and grouped mice are shown in Table 1. Urinary levels of PEA, *m*-TA, PAA, *m*-HPA and *p*-HPA, and plasma levels of PAA and *p*-HPA were significantly lower in isolated aggressive mice (less than 60% of controls in all cases, see Fig. 1). Similarly, the brain levels of PEA and *p*-TA and their major acid metabolites, PAA and *p*-HPA, were lower in isolated mice, although these differences did not achieve statistical significance. In contrast, the brain levels of *m*-TA and *m*-HPA and the plasma levels of *m*-HPA tended to increase in isolated animals (see Fig. 1). The plasma concentrations of PEA, *m*-TA and *p*-TA were below the limits of detection.

A Spearman's Rank Order Correlation Coefficient revealed no significant correlation between intensity of aggression (based on ratings) and concentrations of trace amines and trace acids in isolated mice. However, there was a significant positive correlation between the levels of PAA and *m*-HPA in the plasma and urine of isolated mice (r=.87 and r=.96 respectively, $p<0.002$). Similarly, there was a positive correlation between the urinary levels of *m*-TA and *p*-TA, and *m*-HPA and *p*-HPA in isolated mice (r=.89, and r=.72 respectively, $p<0.05$).

DISCUSSION

The data obtained in this study indicate that, after fighting, isolated aggressive mice exhibit reduced levels of some unconjugated trace amines and their respective unconjugated acidic metabolites compared to group-housed controls. This may indicate a reduced trace amine synthesis and turnover. This finding is at variance with an earlier report by Sandler *et al.* [13] who claimed an increase in PAA levels in the plasma of violent prisoners, suggesting that PEA may be overproduced in aggressive psychopaths. That this may not be a species difference is indicated by a recent study [2] which found a significant decrease in *p*-HPA levels, but only a slight, non-significant, increase in PAA levels in the plasma of offenders housed in a maximum security institution and defined as violent or non-violent on the basis of their court records.

There are, however, a number of factors which severely limit direct comparison of the present findings to data obtained from studies involving prison inmates. Perhaps the most important of these potentially confounding variables is the failure of the present study to distinguish between stress and aggression. It is well established that social isolation causes a variety of other behavioural changes (including stress) in addition to aggression [18,19]. In the present study, each isolated animal was subjected to the additional stress of being housed with two other previously isolated animals for a 24 hr period. Therefore, it is important to note that the observed changes in trace amine and acid levels may be related to increased aggression, increased stress, or, perhaps and most probably, a combination of both factors. In this regard, the present data (see Fig. 1) are in agreement with the observation that aggregation or cold stress in mice produces a decrease in *p*-TA levels and an increase in *m*-TA levels in the striatum [10]. A further limitation is the inadequate definition of aggression, as violence in the prisoner's court-records, which has been used in the recent clinical studies [2,13].

Although the changes in the brain levels of trace amines and trace acids were generally consistent with those observed in plasma and urine (with the exception of *m*-TA and its metabolite *m*-HPA), the effects in the brain were much smaller in magnitude and did not achieve statistical significance. Since it is known that the distribution of these compounds in the brain is heterogenous [3], however, it appears possible that a regional analysis of the neurochemical effects of social isolation on brain trace amine and acid levels might

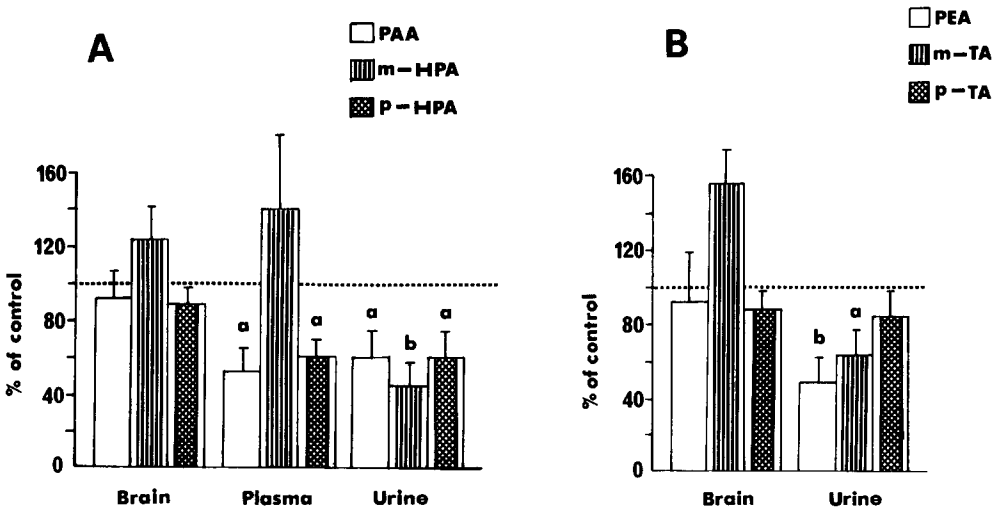

FIG. 1. (A) The concentrations of unconjugated PAA, *m*-HPA and *p*-HPA in the brain, plasma and urine of isolated mice. Results are expressed as percentage of control. Letters refer to statistically significant differences between isolated mice and controls determined by 2-tailed *t*-test for independent means: a, $p<0.01$; b, $p<0.001$. (B) The concentrations of PEA, *m*-TA and *p*-TA in the brain and urine of isolated mice. Details are as described for (a).

reveal significant effects. It should be noted that the significant decrease observed in urinary levels of *m*-TA and *m*-HPA in isolated mice contrasted with the effect on brain and plasma levels of *m*-TA and *m*-HPA which tended to increase. A possible explanation of this discrepancy may be the reported difference in the peripheral and central metabolism of the tyramines [9].

The present data could be interpreted as evidence that reduced levels of PEA and PAA may be related to aggression and fighting in isolated mice. This interpretation is consistent with a report that systemic injections of PEA inhibit the mouse killing behaviour of rats in a direct dose-dependent manner [1].

In summary, our results indicate that unconjugated trace amine and trace acid levels are altered after fighting in isolated aggressive mice compared to group housed control mice of the same strain. Further studies including a regional analysis of the concentrations of brain trace amines, catecholamines, 5-HT and their oxidatively deaminated metabolites are in progress to determine whether the observed neurochemical changes are functionally related to aggression, stress or other components of the isolation syndrome.

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