Monoamines in Brain and Urine of Rats with Hereditary Hypothalamic Diabetes Insipidus

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Summary. Monoamine levels in brain and urine of homozygous and heterozygous diabetes insipidus (DI) rats (Brattleboro strain) were assessed. Homozygous DI rats had a higher whole brain content of serotonin than their heterozygous littermates. However, when corrected for differences in brain weight, homozygous DI also appeared to have higher brain concentrations of noradrenaline, tyrosine and GABA. The total 24 h excretion of all amines and their precursors was greater in the homozygous than in the heterozygous rats.

Vasopressin is involved in the consolidation and/or retrieval of learned responses[4,5], as suggested by animal studies in which learning deficits induced by hypophysectomy were corrected by vasopressin[6]. Recently, a new model, i.e., rats with hereditary hypothalamic diabetes insipidus (DI), has been used to study the behavioural effects of vasopressin. Homozygous DI rats of the Brattleboro strain lack the ability to synthesize vasopressin whereas their heterozygous littermates have a relatively normal water balance[7,8]. Homozygous DI rats are deficient in acquiring a simple passive avoidance response when compared to their heterozygous littermates[9]; this deficient behaviour can be restored by treatment with vasopressin[5].

Nothing is kown about the neurochemical differences between homozygous and heterozygous DI rats. Since the observed behavioural differences may be related to neurochemical differences, we undertook a study to assess monoamine levels in brain and urine of homozygous and heterozygous DI rats. 19 male homozygous and 20 male heterozygous Brattleboro rats were obtained from TNO-Zeist, The Netherlands. These animals were of the same age but body weight was lower in the homozygous rats. At the start of the experiment, the homozygous DI rats weighed approximately 200 g and the heterozygous rats approximately 250 g.

Table I. Excretion of biogenic amines and their precursors in the urine of rats homozygous and heterozygous for hereditary hypothalamic diabetes insipidus

Amine/amino acid	Total concentration			
	Heterozygotes		Homozygotes	
	µg/24 h	µg/ml	µg/24 h	µg/ml
Noradrenaline	0.73	0.05	2.87[a] (+160, +480)	0.03[a] (−59, −25)
Dopamine	1.21	0.10	2.39[a] (+1, +300)	0.02[a] (−88, −69)
Serotonin	8.51	0.50	41.90[a] (+168, +804)	0.34 (−63, +27)
Tyrosine	28.0	1.97	493.0[a] (+1,390, +1,980)	4.28[a] (+70, +180)
Tryptophan	56.8	4.00	1,120.0[a] (+1,650, +2,130)	9.66[a] (+90, +217)

Each value represents the mean of 19 determinations. The percentage change relative to the heterozygous group is also shown, together with the 95% confidence limits (in parentheses). [a] Significance of difference between homozygous and heterozygous group $p < 0.05$.

The rats were housed individually in metabolic cages. After an adaptation period of 1 week, urine was collected for a 24 h period commencing at 10.00 h. The volume of urine was determined and the levels of tryptophan[10], tyrosine[11], serotonin[12], noradrenaline and dopamine[13] were assessed fluorometrically.

After collection of urine, the rats were killed by decapitation. Their brains were rapidly removed and placed on ice. The cerebellum and pineal gland were discarded. The remainder of the brain was homogenized in a dilute acid medium (8 ml 0.01 *N* HCl containing 0.5 ml 10% ethylene diamene tetra-acetic acid as the disodium salt). After centrifugation (800 × *g* for 20 min at 4 °C), aliquots of the supernatant were taken for the estimation of tryptophan[10], tyrosine[11], serotonin[12], noradrenaline and dopamine[13]. An assessment of the concentration of γ-aminobutyric acid (GABA) was also made by means of the fluorometric assay procedure of Uchida and O'Brien[14].

For the statistical analysis of the results, a randomized block analysis of variance was employed. The level of significance used was $p < 0.05$. The 24 h urine volume of the homozygous DI rats was markedly elevated in comparison to that of the heterozygotes (mean 24 h urine volume: 116.0 and 14.3 ml, respectively). The differences between homozygous and heterozygous Brattleboro rats with respect to levels of monoamine and amino acid precursors in the urine are shown in Table I. The total 24 h excretion of all amines and their precursors was significantly greater in the homozygous than in the heterozygous rats. However, when the results were expressed in terms of the concentration of amine or amino acid per ml urine, it appeared that homozygous rats had significantly

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lower concentrations of catecholamines though still significantly higher concentrations of tryptophan and tyrosine than the heterozygotes. The concentration of serotonin did not differ between the groups.

The wet weights of the brains from the homozygous DI rats were less than those of the heterozygous group (mean weight: 1.44 and 1.54 g, respectively).

Table II. Content of biogenic amines, their precursors and γ-aminobutyric acid in the brains of rats homozygous and heterozygous for hereditary hypothalamic diabetes insipidus

Amine/amino acid	Heterozygous		Homozygous	
	Total	Concentration (μg/g)	Total	Concentration (μg/g)
Noradrenaline	1.03	0.66	1.04 (−7, +9)	0.73[a] (+1, +16)
Dopamine	1.85	1.20	1.82 (−10, +5)	1.24 (−8, +16)
Serotonin	0.90	0.58	1.03[a] (+4, +26)	0.72[a] (+11, +37)
Tyrosine	14.88	9.64	15.56 (−6, +16)	10.84[a] (0, +26)
Tryptophan	3.60	2.34	3.34 (−18, +4)	2.32 (−11, +12)
GABA	702	453	723 (−1, +7)	504[a] (+7, +15)

Values are the mean of 19 determinations. Concentrations are given as μg/g wet weight of whole brain. See further legend to Table I.

The brain levels of monoamines, their precursor amino acids and GABA are given in Table II. Homozygous DI rats had a significantly higher whole brain content of serotonin but did not differ with respect to whole brain content of noradrenaline, dopamine, tyrosine, tryptophan and GABA. However, in terms of the concentration of amine or amino acid per g wet weight of the brain, homozygous DI rats appeared to have higher brain concentrations of noradrenaline, serotonin, tyrosine and GABA than their heterozygous littermates.

In conclusion, the present results indicate that there are marked differences between homozygous DI and heterozygous Brattleboro rats with respect to brain and urine levels of monoamines and their precursor amino acids. There is no clear interpretation of these neurochemical differences as seen in relation to the vasopressin deficiency of homozygous DI rats. The sustained levels of activity in homozygous DI rats due to the frequent bouts of drinking may produce changes in amine turnover rates. Furthermore, the high urine content of monoamines and their precursor amino acids in homozygous DI rats may either be due to an increased filtration or excretion or a reduced resorption in the kidney. In addition, the vasopressin deficiency is not the only endocrine anomaly in these rats: the oxytocin content of pituitaries of homozygous DI rats, but not heterozygous rats is greatly reduced, although synthesis of oxytocin is unimpaired[15]. It is not known whether this phenomenon bears any relevance to the findings of the present experiment.

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A Paradoxical Concentration Effect in the Toxicity of Fentin Acetate for Insects

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Summary. Triphenyltin (fentin) acetate residues on glass resulting from the evaporation of acetonic solutions, turned out to be less toxic on contact and finally non-toxic to houseflies and *Spodoptera littoralis* larvae with rising concentration. This paradoxical concentration effect may be due to polymerization of the compound in concentrated solutions.

Under specific conditions, certain compounds may be biologically more active, e.g. more toxic, at lower than at higher concentrations. Such so-called 'paradoxical concentration effects', which seem to be wide-spread in various biological systems, have been reviewed repeatedly by SCHATZ et al.[2]. We wish to report on an easily-reproducible paradoxical toxic effect of fentin (triphenyltin) acetate (henceforth abbreviated to FA) in insects. FA is an agricultural fungicide[3] possessing insect antifeedant[4] properties, but it is only weakly insecticidal in the conventional sense[5].

The toxicity of FA residues for the housefly was assayed by a short-term tarsal contact method[6]: 1.37 ml acetonic solutions of FA[7] of various concentrations (w/v) were introduced into 150 ml round glass jars (inner wall surface area, 137 cm^2), which were then rolled so that a uniform layer formed on their inner surface after the evaporation of the solvent. By this procedure the value of the percentage concentration of the applied solution is the same number as g/m^2 of substance ultimately deposited. 24 h later, 2–3-day-old housefly (*Musca domestica vicina* Macq.) females, immobilized with cold anaesthesia, were introduced into the jars, 10 per jar; there were 4 replications per concentration in an experiment, and each experiment was repeated 4–8 times. The flies were allowed

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