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STRAIN DIFFERENCES IN UPTAKE, POOL SIZE AND TURNOVER RATE OF NOREPINEPHRINE IN HEARTS OF MICE

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Abstract—The uptake, endogenous pool size and turnover rate of norepinephrine were measured in hearts from nine strains of mice. Significant strain differences were noted in each parameter. Catecholamine uptake and endogenous pool size were correlated.

DL-NOREPINEPHRINE-7-^{3}H has been employed effectively as a tool to investigate the uptake of catecholamines by various tissues. After an intravenous (i.v.) injection of DL-norepinephrine-7-^{3}H in cats, Whitby *et al.*[1] and Hertting *et al.*[2] noted a rapid decline in radioactivity of plasma, with a subsequent accumulation of the labeled compound in various tissues including the heart. Observations by Dengler *et al.*[3–5] and Montanari *et al.*,[6] demonstrating accumulation of norepinephrine in tissues against a concentration gradient, suggested that uptake of catecholamines is a process requiring active transport. Uptake and binding of norepinephrine contribute substantially to termination of its action.[7] The endogenous pool of norepinephrine may be considered to be regulated by rates of its synthesis and degradation.

The present study was designed to investigate strain differences in the turnover rates of norepinephrine in hearts from nine strains of mice to determine whether significant genetic differences exist in this and closely related parameters. Endogenous pool size and uptake were also estimated in an attempt to identify possible interrelationships among various factors responsible for catecholamine turnover.

MATERIALS AND METHODS

Adult, male mice of the species *Mus musculus* weighing 20–23 g from the following strains: BALB/c, C3H/HeN, AL/N, DBA/2N, CDF_1, CAF_1, NIH, GP and CFW, were obtained from the NIH animal production unit in Bethesda, Md. Prior to use, all mice were kept six in a cage in the animal quarters of our laboratory on Purina chow and water *ad lib.* and on hard wood bedding in order to maintain environmental conditions as uniform as possible for all strains. DL-Norepinephrine-7-^{3}H, with a specific activity of 9·71 mc/m-mole, was obtained from New England Nuclear Corp., Boston, Mass. At various intervals after DL-norepinephrine-7-^{3}H (5 μc in 0·2 ml of heparinized saline) was injected into the tail vein, mice were sacrificed by cervical dislocation and turnover rates of norepinephrine were determined as described below. The hearts were immediately excised, trimmed of connective tissue, washed, blotted,

weighed and homogenized with 10 vol. of 0·4 N $HClO_4$. The homogenates, allowed to stand in ice for 30 min, were then centrifuged. A 0·3-ml aliquot of the supernatant was dissolved in 15 ml of modified Bray's solution [BBOT (2,5-bis-(5′-tertbutylbenzoxozolyl(2′)-thiophene), 4 g; toluene, 600 ml; methylcellosolve, 400 ml; and naphthalene, 80 g] and counted on a Packard model 3375 TriCarb scintillation spectrophotometer at approximately 15 per cent efficiency. Fluorometric determinations of norepinephrine followed by counts of the radioactivity present in each sample were performed for both control animals not receiving radioactive norepinephrine and for animals given the labeled compound; 30 hr after injection of the labeled catecholamine, 90–92 per cent of the radioactivity was assayed as norepinephrine. The resulting turnover curve was biphasic in all strains studied, as previously described,[6] but the turnover rate was measured using only the second, slower slope, which is the slope that represents norepinephrine turnover.[2]

In the uptake portion of the study, mice injected i.v. with 0·4 μc DL-norepinephrine-7-^{3}H were sacrificed by cervical dislocation 15 min later, and the hearts treated as described previously. Therefore, for the purposes of this study, uptake is defined as the amount of radioactive norepinephrine in cardiac tissue 15 min after intravenous injection of 0·4 μc DL-norepinephrine-7-^{3}H. The lower dose for this portion of the investigation gave a monophasic decline with a half-life approximately equal to the second slope of the biphasic curve described above.

In the final part of the study, endogenous pools of norepinephrine were measured by the fluorometric method of Laverty and Taylor[8] as modified by Leitz *et al.** The alumina used in this assay was washed according to the procedure of Anton and Sayre.[9] Hearts were homogenized with 15 vol. of 0·4 N $HClO_4$ solution containing 0·1% EDTA and 0·25% Na_2SO_3.

RESULTS

Norepinephrine pool in mouse heart. Table 1 shows the endogenous norepinephrine content of hearts from nine strains of mice. Thirty mice from each strain were used for this determination. The norepinephrine content of cardiac tissue ranged from

TABLE 1. POOL HALF-LIFE, POOL SIZE, TURNOVER AND UPTAKE OF DL-NOREPINEPHRINE-7-^{3}H IN HEARTS OF MICE OF VARIOUS STRAINS, WITH RATIO OF HEART TO BODY WEIGHT†

Strain	NE pool half-life (hr)	Pool size (ng NE/ g heart)	NE turnover (ng NE/g heart/hr)	NE uptake (mμmoles NE)	Heart wt. (mg)/ body wt. (g)
BALB/c	12·0	1506 ± 215	87·0 ± 12·4	0·254 ± 0·046	3·97 ± 0·15
C3H/HeN	5·5	934 ± 151	117·7 ± 19·0	0·214 ± 0·026	3·57 ± 0·26
AL/N	9·5	798 ± 132	58·2 ± 9·6	0·126 ± 0·017	4·07 ± 0·18
DBA/2N	8·0	882 ± 130	76·4 ± 11·2	0·172 ± 0·031	4·72 ± 0·24
CDF_1	8·0	984 ± 176	85·2 ± 15·2	0·167 ± 0·022	4·24 ± 0·22
CAF_1	11·0	1052 ± 165	66·3 ± 10·4	0·205 ± 0·015	4·44 ± 0·32
NIH	9·5	758 ± 142	55·2 ± 10·4	0·139 ± 0·016	3·67 ± 0·26
GP	8·0	783 ± 138	67·8 ± 12·0	0·143 ± 0·034	3·50 ± 0·23
CFW	8·0	834 ± 161	72·2 ± 13·9	0·144 ± 0·024	3·84 ± 0·22

† Values are expressed ± S.D.

* F. H. Leitz, F. J. E. Stefano and B. B. Brodie, in preparation.

798 ± 132 ng/g of heart in the AL/N strain to 1506 ± 215 ng/g heart in the BALB/c strain. Hybrid CDF_1 and CAF_1 mice had cardiac norepinephrine levels intermediate in value to the amine content of their parent strains, BALB/c females and DBA/2N and AL/N males, respectively. After the norepinephrine pools were labeled by an injection of DL-norepinephrine-7-^{3}H, the radioactive compound disappeared exponentially with time. Figures 1 and 2 show the decline of DL-norepinephrine-7-^{3}H levels

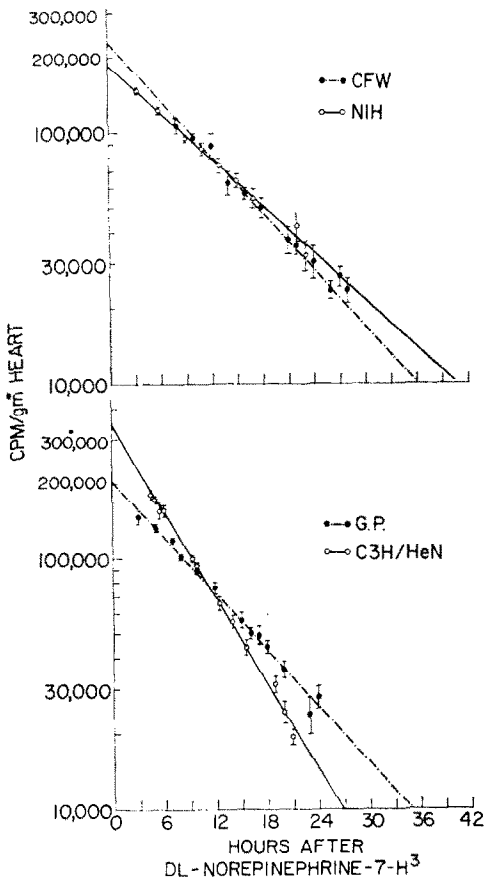

FIG. 1. Decline of DL-norepinephrine-7-^{3}H in hearts from CFW, NIH, GP and C3H/HeN mice in hours after its i.v. injection at time 0. Each point represents the mean for at least ten mice with the standard deviations indicated by bars above and below each point.

in hearts from nine strains of mice. The half-life of the norepinephrine pool for each strain was estimated from this plot. K_p, the slope of the exponential curve, was determined from the following equation: $K_p = 0{\cdot}693/T_{\frac{1}{2}}$. Values for the pool half-life ranged from 5·5 hr in C3H/HeN mice to 12·0 hr in BALB/c mice (Table 1). No significant correlation existed between the endogenous norepinephrine content of heart tissue and the pool half-life of norepinephrine ($P > 0{\cdot}05$). Although significant strain differences existed for the half-lives of the cardiac pool of norepinephrine,

preliminary studies revealed no significant sex differences in pool half-life within strains.

Turnover rate of norepinephrine in mouse heart. For each strain, the turnover rate of cardiac norepinephrine was determined from the equation: turnover rate $= K_p \times N$, where N is the norepinephrine content expressed in nanograms of norepinephrine per gram of heart. Table 1 shows that the turnover rate varied from 55·2 $\pm$ 10·4 ng NE/g heart/hr in NIH mice to 117·7 $\pm$ 19·0 ng NE/g heart/hr in the C3H/HeN mice.

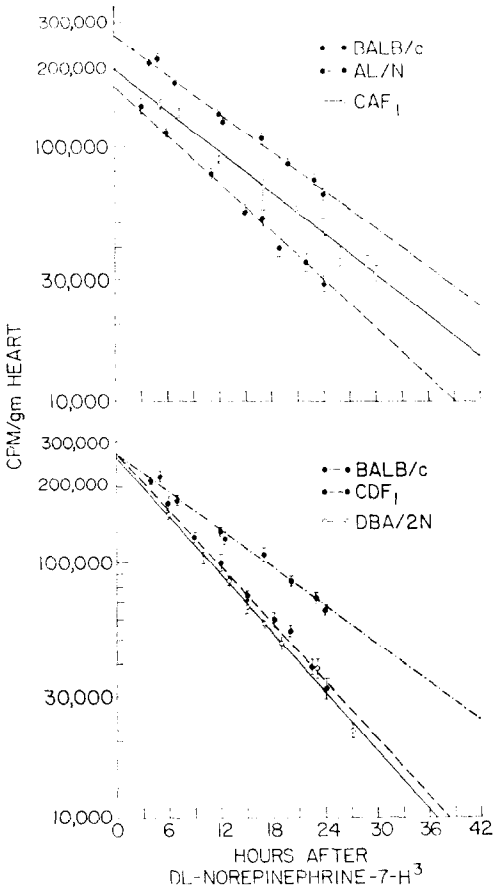

FIG. 2. Decline of DL-norepinephrine-7-^{3}H in hearts from BALB/c, AL/N, CAF_1, CDF_1 and DBA/2N mice in hours after its i.v. injection at time 0. Each point represents the mean for at least ten mice with the standard deviations indicated by bars above and below each point.

Uptake of DL-*norepinephrine-7-*^{3}*H by mouse heart.* Table 1 reveals that the uptake of DL-norepinephrine-7-^{3}H ranged from 0·126 mμmoles norepinephrine in the AL/N strain to 0·254 mμmoles norepinephrine in BALB/c mice. Thirty mice from each strain were used to determine the values reported in Table 1. Although no significant correlation existed between uptake of norepinephrine by heart tissue and turnover rate of cardiac norepinephrine ($P > 0·05$), a statistically significant correlation

between cardiac norepinephrine content and uptake of norepinephrine was noted ($P < 0.01$). A correlation coefficient of 0·89 was obtained for these two parameters.

The ratio of heart weight to body weight ranged from 3·50 ± 0·23 mg heart/g body weight in GP mice to 4·72 ± 0·24 mg heart/g body weight in the DBA/2N mice. Thirty mice from each strain were used to determine these values. No significant correlation was observed between the ratio of heart to body weight and the pool size, turnover rate or uptake of norepinephrine in cardiac tissue ($P > 0.05$ for each correlation coefficient).

DISCUSSION

In adrenergically innervated tissues such as heart, sympathetic tone depends on the amount of free norepinephrine available at adrenergic receptors. Even under steady state conditions, the free amine is in a dynamic state of flux, simultaneously being synthesized and removed by either enzymatic degradation or reuptake at sympathetic nerve endings.

The results of the present investigation reveal statistically significant differences among nine mouse strains in the half-life of the endogenous pool of norepinephrine. These differences reflect variation in the utilization of norepinephrine. Table 1 shows three groups of mice: C3H/HeN mice have a short half-life for the cardiac norepinephrine pool, whereas BALB/c and CAF_1 mice have relatively long pool half-lives. The remaining six strains comprise a third group with half-lives for their cardiac norepinephrine pool intermediate between those of the C3H/HeN strain and the BALB/c and CAF_1 strains. Differences in utilization of norepinephrine among nine mouse strains could be either genetic or environmental in origin. As described under Materials and Methods, care was devoted to maintaining the environmental conditions of all mice as uniform as possible. We therefore favor the interpretation that our results suggest genetic differences among mouse strains in uptake, pool size and turnover rate of cardiac norepinephrine.

In mouse heart, norepinephrine pool size correlated with uptake of exogenous norepinephrine. Differences in uptake of exogenous norepinephrine could reflect differences in number of neurons or in number of uptake sites on the neurons. Alternatively, the different strains of mice may have similar numbers of uptake sites, but may vary in ability to transport the amine across a concentration gradient from plasma to neuron. Strain differences in uptake were identified, but this study does not permit identification of mechanisms responsible for the correlation between norepinephrine uptake and the size of the endogenous pool of labeled amine.

Since norepinephrine turnover is a function of both the norepinephrine pool size and pool half-life, our results reflect the rate of norepinephrine synthesis in mouse heart. Enzymatic degradation of norepinephrine within cardiac neurons is negligible, as shown by our observation that 90–92 per cent of the radioactivity in mouse heart at 30 hr after catecholamine injection was norepinephrine. A comparison of C3H/HeN and CDF_1 mice shows that these two strains have similar pool sizes and uptakes for norepinephrine, but different pool half-lives. These results suggest dissimilar rates of norepinephrine synthesis in the hearts of C3H/HeN and CDF_1 mice. C3H/HeN mice exhibit a shorter norepinephrine pool half-life than CDF_1 mice and would be expected to synthesize norepinephrine at a faster rate than the CDF_1 mice.

For norepinephrine pool size, pool half-life, turnover and uptake, as well as for

ratio of heart to body weight, the hybrid strains CAF_1 and CDF_1 exhibited values intermediate to those of their parents, AL/N and DBA/2N males, respectively, and BALB/c females. Because graded or metrical characters are frequently determined by alleles at multiple loci,[10] these results suggest that such parameters may be under polygenic control.

The results of this study reveal differences in utilization and synthesis of norepinephrine among nine strains of mice. Additional strain variations in such physiological parameters as blood pressure may exist and perhaps may be related to the strain differences in norepinephrine kinetics that we have described. C3H/HeN mice were noted by us to be much more active and aggressive than BALB/c mice. Although this difference in behavior may be related to variations between these strains in rate of norepinephrine synthesis, it may be coincidental, since behavior is more a function of central activity, whereas turnover of norepinephrine as measured in these studies occurred in a peripheral tissue. However, correlations have been reported between endogenous norepinephrine levels in brain from various mouse strains and strain differences in patterns of behavior.[11-12]

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