

Blood pressure in rats: a comparison of a multifactorial experimental design to measurements in an outbred stock

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Summary

Systolic blood pressure was measured in males of 8 inbred strains and 1 outbred stock of rats 5 times over a period of 20 min on 5 consecutive days. The strain means ranged between 107.9 mmHg and 149.3 mmHg. The estimated variance between strains ($V = 248.7$ mmHg) was about 5 times higher than the variance within strains ($V = 54.3$ mmHg). The intraindividual variance within strains was relatively constant ($V = 24.0-37.6$ mmHg), while the interindividual variance varied to a great extent ($V = 4.5-44.5$ mmHg) from strain to strain. The outbred stock showed values of blood pressure and components of variance similar to those of a single inbred strain. Thus, by investigation of a battery of 8 inbred strains in a multifactorial experimental design a greater phenotypic variability due to genetic strain differences is achieved than by measurements in a single outbred stock.

The variability within inbred strains represents exclusively the non-genetic variance component within as well as between animals. In contrast, the variability within an outbred stock should be caused by both the genetic and environmental components. This is the main justification for the use of outbred stocks in toxicological screening tests, where a wide range of phenotypes due to individual genetical differences is demanded. In contrast to this Festing (1979b) suggested sampling from several inbred strains in a multifactorial experimental design because a wider spread of phenotypes can be achieved than by sampling from a single inbred strain. Such a design should also give a wider range of phenotypes than sampling from an outbred stock.

There is evidence that the variability in systolic blood pressure between different inbred strains of rats is several times higher than within strains (Hansen, 1972). Some inbred strains of rats, such as SHR, WKY and GH (Okamoto, 1969; Phelan *et al.*, 1972) are known to have been systematically selected for high or low blood pressure, while most other inbred strains are selected exclusively for criteria which are not directly linked to blood

pressure. The heritability of blood pressure in a broad sense was estimated by Tanase & Suzuki (1971) to be $d^2 = 0.64$. Little is known about the variability of blood pressure in outbred laboratory stocks.

The aim of the present study was to demonstrate that for blood pressure, sampling from several inbred strains gives a much broader spread of phenotypes due to genetical strain differences than sampling from a single outbred stock.

Materials and methods

Animals

Blood pressure was determined between the 125th and 130th day of age in 6 males of each of the following 8 inbred strains of rats: ACI/Ztm, AS/Ztm, BH/Ztm, BS/Ztm, DA/Ztm, LEW/Ztm (Festing, 1979a, b), MWF/Ztm (Hackbarth *et al.*, 1980) and WC/Ztm (Gärtner, 1969). The animals were reared and kept under SPF conditions (barrier-maintained, free from infections listed by the Society of Laboratory Animal Science, 1977) in polycarbonate cages (Makrolon type III, 14 x 26 x 20 cm). Room temperature was $22 \pm 2^\circ\text{C}$, relative humidity $55 \pm 5\%$, light from 7 a.m. - 7 p.m. Pelleted food containing 19% protein, 4% fat, 0.9% calcium, 0.7% phosphorus, and 0.2% sodium (Altromin 1324, Altromin International, Lage, FRG) and tap water from bottles were available *ad libitum*; wooden granules were used as bedding material.

10 days prior to the experiment the rats were transferred from the breeding unit and were kept individually in the animal room, where the measurements were performed. Each test group consisted of 6 animals of different strains which were selected randomly.

16 male Han:WIST rats were delivered from the Zentralinstitut für Versuchstiere in Hannover at 100-days-old. From their arrival they were kept under identical conditions to those of the inbred strains. The age of these animals at the beginning of test was 110 days for 6 animals, 117 days for another 6 animals and 124 days for 5 animals.

Blood pressure measurements

Systolic blood pressure was measured in restrained conscious animals at 32°C room temperature with a Doppler ultrasonic method described in principle by Buñag (1973). For comparisons between direct and indirect measurements of arterial blood pressure see also Yamakoshi *et al.* (1979). 2 training periods were performed before the final measurements were started. The final blood pressure determinations were made on 5 consecutive days 5 times per session at 10, 15, 20, 25 and 30 min after being restrained by a wiremesh restraining tube and after being transferred to the preheated box at 32°C.

Results

Means and standard deviations of blood pressure (BP) and bodyweight (BW) of the 8 inbred strains and the outbred Han:WIST stock as well as the mean of all inbred strains are listed in Table 1. The strains are ranked according to their blood pressure. LEW/Ztm is the strain with the lowest blood pressure (107.9 mmHg), MWF/Ztm the strain with the highest blood pressure. The mean of all inbred strains is nearly identical to the mean of the Han:WIST stock (126.6 mmHg, 127.5 mmHg respectively). The standard deviations within strains differ from 5.46 (WC/Ztm) to 9.40 (BH/Ztm), with the Han:WIST stock being in the middle of this range (7.48 mmHg). Because of the large differences in blood pressure between the different inbred strains the standard deviation of all inbred rats (ignoring strains) was about twice as large as within a single inbred strain. All strain differences in mean blood pressure were significant at the 0.1% probability level according to Student's *t*-test with the exception of WC/Ztm versus DA/Ztm (n.s.) and AS/Ztm versus MWF/Ztm ($P < 5\%$).

Bodyweight varied from 245.0 g in the DA/Ztm strain to 387.5 g in the LEW/Ztm strain. There was no correlation between blood pressure and bodyweight. An analysis of variance of blood pressure in a 3-factor design with the factors of

strain, between days and within days revealed highly significant differences between strains ($F = 690.88$, $DF = 7; 11$, $P < 0.01$) and interactions of strain and between days ($F = 4.47$, $DF = 28; 112$, $P < 0.01$). There were no significant differences or trends within trials. In some strains (AS/Ztm, BH/Ztm, WC/Ztm) small but significant differences or trends from day to day existed. These interactions between strains and days were neglected for further calculation.

The components of variance between and within animals were compared separately for each strain (Table 2). Because the values are not totally normally distributed there is a slight difference of the sum of variances between and within animals compared to the total variance. Between 14.4% (WC/Ztm) and 62.6% (DA/Ztm) of the variability within strains is due to the variability between animals. The *F*-ratios of the variances between animals between DA and BH to WC are significant with $P < 0.01$, those of LEW, BS, WIST and MWF to WC with $P < 0.05$. The mean of all inbred strains (49.8%) is close to the one of the outbred Han:WIST stock (45.0%).

An estimation of the components of variance within and between inbred strains by a 2-factorial analysis of variance gives a variance between strains of $V = 248.74$, between animals of $V = 6.06$, between strains*animals of $V = 23.34$ and a residual (within animals) of $V = 29.67$. These results are in good agreement with the results listed in Tables 1 and 2. Compared to the variability within strains, the variability between strains is about 5 times higher and differs therefore significantly from the variability within strains ($F = 4.9$, $DF = 47.16$, $P < 0.01$).

The heritability in a broad sense estimated by a simple intraclass correlation (Festing, 1979) was $r_1 = 0.56$.

Discussion

The variation of systolic blood pressure between

Table 1. Blood pressure (BP) and bodyweight (BW) (mean and SD) of different strains of rats

	LEW/Ztm	BS/Ztm	ACI/Ztm	WC/Ztm	DA/Ztm	Han:WIST	BH/Ztm	AS/Ztm	MWF/Ztm	Mean of all inbred animals
BP \bar{X}	107.85	112.36	117.08	124.23	124.23	127.52	136.17	147.55	149.27	126.56
BP SD	7.18	7.11	7.02	5.46	8.72	7.48	9.40	5.85	7.42	16.60
				└ n.s. ─				└ $P < 0.05$ ─		
BW \bar{X}	387.5	333.8	254.4	334.9	245.0	341.7	347.9	303.3	361.3	321.0
BW SD	23.4	15.9	9.8	17.4	15.3	30.4	19.3	18.1	24.1	50.4

Means are ranked from low to high for blood pressure.

Table 2. Components of variance of blood pressure in different strains (absolute values and %)

	<i>LEW/Ztm</i>	<i>BS/Ztm</i>	<i>ACI/Ztm</i>	<i>WC/Ztm</i>	<i>DA/Ztm</i>	<i>Han: WIST</i>	<i>BH/Ztm</i>	<i>AS/Ztm</i>	<i>MWF/Ztm</i>	Mean of all inbred strains
Between animals	32.30	25.94	13.57	4.49	52.97	25.85	60.45	12.12	33.46	29.40
		* -----			** -----		* -----			
Within animals total	24.40	28.75	37.83	26.08	31.62	31.62	37.55	24.04	27.04	29.66
	51.50	50.53	49.22	29.77	76.06	56.00	88.27	34.21	55.10	54.33
% between animals	57.0	47.4	26.4	14.4	62.6	45.0	61.7	33.5	55.3	49.8
% within animals	43.0	52.6	73.6	85.6	37.4	55.0	38.3	66.5	44.7	50.2

Variance between and within animals and total variance were estimated by analyses of variance.

the strains used in the present study is in good agreement with the variance reported by Hansen (1972). Our results support his opinion, that inbred strains of rats, which are not selected for blood pressure, show a tendency to be distributed randomly. Hansen (1972) found the lowest systolic blood pressure in the ACI/N strain (116 mmHg) and the highest in the OM/N strain (160 mmHg) when the SHR/N strain, which has been selected for high blood pressure (Okamoto, 1969), is excluded. In our study the strain LEW/Ztm (108 mmHg) had the lowest and the MWF/Ztm strain (148 mmHg) the highest systolic blood pressure. Systolic blood pressure in the BS strain reported by Phelan *et al.* (1972) differs less than 1 mmHg from our value. Moreover, the AS strain, which is closely related to the GH strain (Heslop & Phelan, 1973) also shows a high blood pressure. These findings and nearly identical values of the ACI strain measured by Hansen (1972) and by us confirm the good reliability of the method used and show that sublimes of inbred strains do not differ markedly in blood pressure. This variability between strains is comparable to that of human populations (Ambrosio *et al.*, 1976; Brussard *et al.*, 1981).

While within inbred strains the estimated variance between animals differs from $V = 4.49$ in the strain WC to $V = 60.45$ in the BH strain, the variance within animals is relatively constant ($V = 24.04-37.83$). Only the strain WC differs significantly from others, but this may in part be due to the small number of animals/strain used in our study, but a genetic determination of the interindividual variance as suggested again by Hansen (1972) cannot be excluded. Such a genetic determination of variance within inbred strains has also been reported for several variables in rats and mice (Gärtner *et al.*, 1976; 1978).

Standard deviations of systolic blood pressure from several different studies vary between 1 to 16 mmHg (Franklin *et al.*, 1982; Yen *et al.*, 1974, Orlov *et al.*, 1982; Ten Berg, 1980; Rupp & Jakob, 1981) independently of the genetic status of the animals used. Generally the standard deviations of different strains within one study do not differ to the same extent as the standard deviations

between different studies. These differences are mainly due to differences in the experimental design as well as the different methods used, such as ambient temperatures during the measurements (Yen *et al.*, 1978).

Although genetic theory proposes that outbred stocks should be more variable, the statistical demonstration of this requires large sample sizes due to the large sampling variance of a variable. Nevertheless, our findings do not support the hypothesis of a greater variation of blood pressure within outbred stocks in comparison to inbred strains. Yen *et al.* (1974) using well defined animals found nearly identical standard deviations within the outbred SDR stock as within the inbred WFR strain. This agrees with our finding, that the total variance within the outbred Han:WIST stock is nearly the same as the mean of the variances within the different inbred strains tested. Moreover, the components of variance between animals, within animals and the mean of systolic blood pressure are nearly identical. Thus, the outbred Han:WIST stock represents the mean of all inbred strains used in this study. The genetic variability of outbred stocks will differ according to their genetic history and amount of inbreeding. A possible screening of several outbred stocks may reveal stocks with higher variability. There is likely to be considerable overlap in the amount of intrastain or stock variability. We have examined only a single outbred stock in this study, but on the basis of the data presented a single inbred strain gives a range of phenotypes as wide as in the Han:WIST outbred stock. Thus there is evidence that sampling from several inbred strains increases the variability to an amount which is not achieved within an outbred stock. Moreover such a multifactorial experimental design, as has also been suggested for toxicological screening (Festing, 1979), allows a clear separation of genetic effects from others and makes experiments repeatable even after several years.

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Blutdruck bei Ratten: ein Vergleich eines mehrfaktoriellen Versuchsansatzes zu Messungen in einem Auszuchtstamm

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Zusammenfassung

An männlichen Ratten von acht Inzuchtstämmen und einem Auszuchtstamm wurde der systolische Blutdruck an fünf aufeinanderfolgenden Tagen jeweils 5 mal über einen Zeitraum von 20 Minuten gemessen.

Die Stammesmittelwerte lagen zwischen 107.9 mmHg und 149.3 mmHg. Die geschätzte Varianz zwischen den Stämmen war mit $S = 248.7$ mmHg etwa fünfmal größer als die mittlere Varianz innerhalb der Stämme ($S = 54.3$ mmHg).

Die intra-individuelle Varianz innerhalb der Stämme ($V = 24.04 - 37.55$ mmHg) war relativ konstant, während

die inter-individuelle Varianz zwischen $V = 4.5 - 44.5$ mmHg je nach Stamm schwankte.

Der Auszuchtstamm zeigte Blutdruckwerte und vor allem Varianzkomponenten die vergleichbar waren mit denen eines einzelnen Inzuchtstammes.

So daß durch Untersuchungen einer Batterie von acht Inzuchtstämmen in einem mehrfaktoriellen Versuchsansatz eine größere phänotypische Variabilität auf Grund genetischer Stammesunterschiede erreicht wird als durch Messungen eines einzelnen Auszuchtstammes.