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Sound-withdrawal hypertension in rats—a new form of experimental hypertension

J. F. MARWOOD AND MARY F. LOCKETT

Department of Pharmacology, The University of Western Australia, Nedlands, Western Australia

Females of an inbred strain of Wistar rats, initially 160–180 g, develop hypertension when transferred from the stock (normal rat) room (NRR) to a sound-proofed semi-anechoic room (SPR). No hypertension develops when sounds in the NRR are relayed unchanged into the SPR. The hypertension reaches maximum at 4 weeks then decreases slightly to stabilize at a level approximately 30 mm Hg above normal before, and 20 mm Hg above normal after, pentolinium blockade. Histological changes are demonstrable in small arteries after 12 weeks in the SPR. Augmented pressor responses to fixed doses of noradrenaline and of tyramine, but not of angiotensin II and of vasopressin, and a general increase in organ/body wt ratios were evident in weeks 3–5 after entry to the SPR, but had reversed by week 12. These findings contrast with those made on salt-DOCA hypertensives.

It is well known that hypertension can be generated in rats by subjecting them repeatedly, for several months, to multiple or to single stressful situations. Loud noises, flashing lights, cage rocking and blasts of air have been administered repetitively, in random order, for periods ranging from 16 to 33 weeks and have produced hypertension (Farris, Yeakel & Medoff, 1945; Hudak & Buckley, 1961; Rosecrans, Watzman & Buckley, 1966). In the absence of other stressors, auditory excitation can also generate hypertension in rats (Medoff & Bongiovanni, 1945) which persist for several months after all exposure to the noises has ceased (Smirk, 1949).

The work now newly reported demonstrates the production of lasting hypertension in rats by withdrawal of all adventitious sounds. The hypertension is fully developed in 6 weeks; histological arteriolar changes are apparent at 12 weeks and by this time, the hypertension is fully established and has become lasting. Some initial studies of the cardiovascular changes in sensitivity to pressor agents which characterize this new form of experimental hypertension are also reported.

METHODS

One hundred and ninety-six female Wistar rats of a single inbred strain were used, initial weight 160–180 g. Before use, all were accustomed to handling and to injections and, when necessary, to metabolism cages and stomach tubes. The rats were housed two per standard cage in a semi anechoic sound-proofed room or in a stock rat room. Both rooms were well ventilated (8 changes h^{-1}) and were maintained at 24°. Illumination was from 6 a.m. to 6 p.m. daily. All rats ate a pellet diet (Wesfarmers Ltd., Australia) containing 0·23 g sodium (Na) and 0·38 g potassium (K) per 100 g, and drank freely. The tap water supplied as drinking fluid was replaced by 0·7% sodium

chloride (w/v) in tap water solely for the production of salt hypertension and DOCA (1 mg per rat, 4 times weekly, s.c.) with salt hypertension.

Mean systemic arterial pressure was measured under anaesthesia induced by pentobarbitone-sodium (45 mg kg^{-1}, i.p.). Polyethylene tracheal, L. external jugular and R. common carotid cannulae were inserted. Mean systemic arterial pressure was recorded from the carotid annula (heparinized) by means of an E & M force-displacement transducer (Model p-1000) coupled to a Mesco pen-recorder (Model JY 110–2). Steady mean arterial pressures were recorded for 5 to 10 min before transmission was blocked in all ganglia of the autonomic nervous system by administration of pentolinium, 1 mg (i.v.) or 5 mg (s.c.) per rat. These doses had been shown to produce maximum and sustained decrease in mean arterial pressure and to block cardiac responses to stimulation of the caudal portion of the divided vagus. After pentolinium the mean arterial pressure fell, stabilized and was again recorded. Pressor responses to submaximally effective doses of (—)-noradrenaline (0·25 μg) tyramine (25 μg), angiotensin II-val^5-amide (1·0 ng) and vasopressin (0·2 mU) were recorded as described by Nicholas (1971). These drugs were administered via the jugular cannula in 0·1 ml isotonic saline.

The semi-anechoic sound-proofed room (*SPR*) had a working space within it of 3·8 × 3·0 × 2·15 m and had double walls made of particle board which were separated by a 43 cm gap. The external outer wall was covered by lead sheeting and the external inner wall was covered with a 5·0 cm layer of high density foam rubber. The floor, ceiling and door were similarly constructed; the door sealed into position, and was approached through a sound-trap. All controlling devices were mounted on panels outside the sound trap. Internal microphones and speakers were also controlled from the panels. Intense intermittent external noises were attenuated to less than 45 decibels (db) in the rat cages within the SPR. The sounds emitted by the rats themselves ranged from 30 db (when sleeping) to 45 db within the cages. A Precision Sound Level Meter (type 2203, Bruel and Kjoer, Copenhagen) was used to measure decibels.

Rats were caged in pairs in twelve open mesh wire cages (38·5 × 23 × 23 cm) above polythene trays, containing sawdust for excreta. The rats remained undisturbed except for approximately 15 min per week during which food and water were replenished and the trays changed with the minimum of disturbance.

Post mortem and histological examinations. Rats were stunned and decapitated. Hearts, spleens, thymus glands, kidneys and adrenals were cleaned of adherent fat and weighed. Portions of mesentery and a gastrocnemius muscle were removed and fixed in Bouin's fluid for histological examination. Sections (6 μm) were stained by Verhoeff's method (1908), differentiated in 2% ferric chloride and mounted in Depex.

Disc electrophoresis of whole pituitary glands was by the method of Ornstein (1964) incorporating the homogenized gland into the large pore sample gel (4·5% acrylamide), stacking the lower gel (7·5% acrylamide) at pH 8·9 and running with tris-glycine buffer, pH 8·3, in the electrode compartments. A current of 5 mA was applied per tube for 30 min. Gels were stained by immersion in 1% Amido-Schwartz reagent for 1 h and were destained electrophoretically in 7·5% acetic acid. The protein bands were examined by means of a double beam recording and integrating densitometer (Joyce, Loebl and Co. Ltd.).

Drugs. (−)-Noradrenaline (Winthrop Laboratories) tyramine hydrochloride (Koch-Light Laboratories Ltd.) angiotensin II-val^5-amide (Ciba) vasopressin (Parke Davis & Co. Ltd.), heparin (Evans Medical), DOCA (Ikapharm, Ramat-Gan, Israel) and methyl prednisolone acetate (Upjohn Company, Kalamazoo), were obtained commercially.

RESULTS

The development of sound-withdrawal hypertension

The changes in mean systemic arterial pressure developed by female Wistar inbred rats in the first 12 weeks after transfer to the sound-proofed room (SPR) are shown in Fig. 1 before (A) and after (B) transmission in autonomic ganglia had been blocked by pentolinium. The hatched areas show the limits of $\pm$ one standard error about the corresponding average values for the mean systemic arterial pressure of rats which had been fed and maintained under similar conditions, in a similarly sized room (NRR) which was not sound-proofed. Mean systemic arterial pressure had risen in rats after 2 weeks in the SPR ($P < 0{\cdot}05$), reached a peak value at the end of 4 weeks and fell slightly to reach a level of 33 mm Hg in excess of normal by the end of week 6. This value was maintained in the SPR, without change, to the end of the 12 weeks. Rats transferred from the SPR to the NRR at 5 weeks showed no abatement of hypertension after a further 7 weeks. Arterioles of those removed at 12 weeks had developed histologically demonstrable hypertrophy of the muscle coat and reduplication of the internal elastic laminae. Mean arterial pressures recorded after blockade of autonomic ganglia were found significantly increased ($P < 0{\cdot}05$) at the end of the 2nd week but had returned to normal by the end of the 3rd week and had risen approximately 15 mm Hg by the end of the 4th week ($P < 0{\cdot}5$) to a value maintained until the 8th week.

By the end of the 12th week in the SPR the mean arterial pressure in the ganglion-blocked animals had risen to 20 mm Hg above control values. The heart rates of rats from the SPR and NRR did not differ.

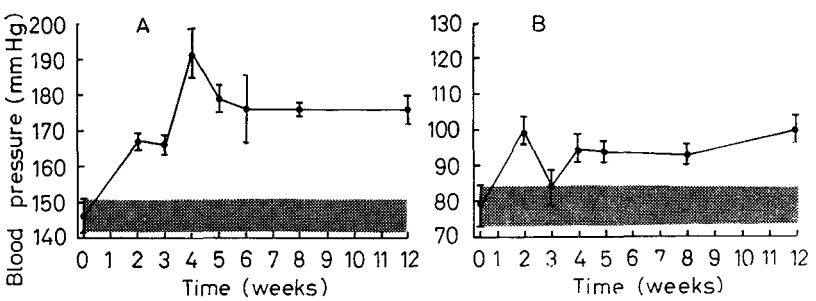

FIG. 1. Time course of the development of hypertension in the females of an inbred strain of Wistar rats caused by transfer to a semi-anechoic sound-proofed room (SPR). Mean systemic arterial pressure is plotted as ordinate against weeks in the SPR as abscissa, before (A) and after (B) induction of ganglion block. Each point is a mean supplied by 8 rats, each vertical is a standard error of the mean. The shaded areas show a range of the mean $\pm$ one standard error for normal female rats of this strain and of comparable body weight.

Rats in the SPR maintained in silence invariably developed hypertension. However, in each of two experiments in which sounds from the NRR were continuously relayed to the SPR at unaltered intensity, no hypertension developed in the SPR animals.

Table 1. *Changes in organ weights (mean ± s.e.) of rats during the development of sound-withdrawal hypertension.*

Time (weeks in SPR)	Body weight (g)	Heart weight (mg)	Thymus weight (mg)	Adrenal weight (mg)	Spleen weight (mg)
Controls (NRR)					
Week 2	185·9 ± 6·9	620·3 ± 18·9	201·9 ± 12·8	30·0 ± 1·3	591·9 ± 42·7
2	193·3 ± 3·6	576·8 ± 19·1	225·0 ± 16·6	29·0 ± 1·3	688·1 ± 37·2
3	186·9 ± 5·4	641·4 ± 21·5	252·8 ± 24·0	33·9 ± 1·4	785·1 ± 39·9
4	193·6 ± 6·8	613·2 ± 15·7	286·5 ± 17·0	31·5 ± 1·3	747·8 ± 28·6
5	203·0 ± 3·4	646·9 ± 23·9	297·4 ± 22·1	32·8 ± 1·2	745·0 ± 24·0
6	209·4 ± 3·8	670·0 ± 13·1	268·4 ± 11·0	32·0 ± 0·8	625·1 ± 16·5
8	194·0 ± 2·7	571·6 ± 11·2	219·4 ± 15·9	26·4 ± 1·0	728·6 ± 46·5
12	219·8 ± 6·6	649·0 ± 16·9	236·4 ± 13·7	29·6 ± 1·3	775·5 ± 65·4
Controls (NRR) Weeks 4 and 5	196·0 ± 5·5	574·6 ± 12·7	227·6 ± 11·7	30·1 ± 2·2	729·2 ± 40·1

The values shown are means ± their standard errors. Not less than 8 animals contributed to each mean. NRR = Stock rat room. SPR = Sound-proofed room.

Body weight increased overall normally in the SPR rats (Table 1). Organ to body weight ratios in general increased in parallel with the blood pressure to become maximal between weeks 3 and 5 (Fig. 1) but then declined to the initial values, whereas the blood pressure remained significantly elevated. The stores of thyrotropin, growth and lactogenic hormones (as determined by quantitative disc electrophoresis) in the anterior hypophyses of rats from the SPR and from the NRR did not differ: neither did the weights of the glands.

Changes in sensitivity to pressor amines found during development of sound-withdrawal hypertension

Responses of the mean systemic arterial pressure to (−)-noradrenaline, tyramine, angiotensin II-val^5-amide and to vasopressin were measured in rats which had been housed in the SPR for periods of up to 12 weeks. These, and corresponding measurements made on animals housed in the NRR, were made under pentobarbitone anaesthesia after induction of full ganglionic blockade by administraion of pentolinium. Fig. 2 summarizes the experimental findings: each point is the mean of observations made of a group of 8 rats. Marked fluctuations in responses to pressor amines were observed during the first but not in the second 6 week period after transfer to the SPR. The pressor effects of (−)-noradrenaline and of tyramine were significantly ($P < 0{\cdot}05$) increased during weeks 3–5 in the SPR (Fig. 2) when mean arterial pressure in the absence of ganglionic blockade was also at peak value (Fig. 1). Thereafter, although mean arterial pressure remained raised (Fig. 1), responses to (−)-noradrenaline and to tyramine, recorded during full pentolinium blockade, returned to normal levels (Fig. 2). Variations in the pressor effects of vasopressin did not reach significance. A just significant ($P < 0{\cdot}05$) increase in the effect of angiotensin II-cal^5-amide appeared in the 12th week.

Comparison of the pressor effects of (−)-noradrenaline, tyramine, vasopressin and angiotensin in ganglion blocked rats, normal and made hypertensive by sound-withdrawal, salt alone, and salt with DOCA

Comparison was made of pressor responses to fixed doses (Table 2) of (−)-noradrenaline, tyramine, angiotensin II-val^5-amide and vasopressin in normal rats and in rats during the 5th week of treatments designed to produce hypertension. These treatments were sound-withdrawal, substitution of 0·7% NaCl for water as sole

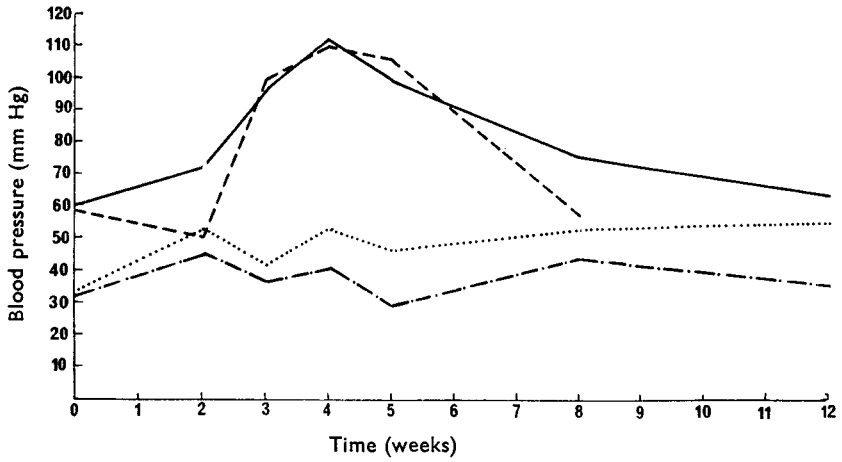

FIG. 2. Time courses of changes in cardiovascular reactivity to fixed i.v. doses of (−)-noradrenaline (——), angiotensin II-val[5]-amide (. . .), tyramine (– – –) and vasopressin (—·—) shown after ganglionic blockade by female rats of an inbred Wistar strain housed in a sound-proofed room (SPR). Ordinates: mm Hg rise in mean systemic arterial pressure. Abscissae: weeks spent in the SPR. Each point is a mean value supplied by 8 rats.

Table 2. *A comparison of the effects of the responses of the mean systemic arterial pressures of ganglion-blocked rats (in various forms of hypertension) to (−)-noradrenaline, tyramine, angiotensin II-val[5]-amide and vasopressin,* i.v.

Types of hypertension	Weight g	Mean systemic arterial pressure, mm Hg		mm Hg rise in mean arterial pressure caused in pentolinium treated rats by various pressor agents			
		Before pentolinium	After pentolinium	(−)-Noradrenaline 0·25 μg	Tyramine 25 μg	Angiotensin 10 ng	Vasopressin 0·2 mU
None (NRR)	194 ± 4·1 (8)	136 ± 4·1	90 ± 2·6	79 ± 4·4	58 ± 2·4	53 ± 2·7	45 ± 2·9
Sound-withdrawl 5th week	194 ± 6·8 (8)	192 ± 4·9**	95 ± 3·8	112 ± 5·2**	110 ± 5·7**	53 ± 3·3	40 ± 3·8
Salt in NRR 5th week	208 ± 7·5 (8)	190 ± 4·7**	105 ± 3·2*	76 ± 2·6	60 ± 2·1	59 ± 1·8	40 ± 1·7
Salt + DOCA in NRR 5th week	208 ± 6·9 (7)	196 ± 3·4**	115 ± 0·8**	83 ± 7·6	59 ± 1·4	64 ± 4·0	34 ± 2·4

The values shown are mean ±s.e. followed by the number of animals contributing to the means in parenthesis.
The significance of differences from means found for untreated rats housed in the normal rat room (NRR) have been examined by Fisher's '*t*' test for small numbers and are indicated by asterisks: one, $P < 0.05$; two, $P < 0.01$.

drinking fluid and the drinking of 0·7% NaCl with additional DOCA, 1 mg per rat (s.c.) four times weekly. All measurements were made under pentobarbitone anaesthesia during blockade of autonomic ganglia produced by injection of pentolinium, 1 mg (i.v.) or 5 mg (s.c.), per rat.

Significant hypertension had been produced in all three groups of treated animals by the fifth week of treatment. Whereas the mean systemic arterial pressures of the rats drinking saline with or without additional DOCA remained significantly above normal values after induction of ganglion-block, those of the animals exposed to sound-withdrawal in the SPR did not (Table 2). Abnormal responses to pressor agents were found solely in the sound-withdrawal hypertensives in which the pressor effects of (−)-noradrenaline ($P < 0.01$) and of tyramine ($P < 0.01$) were significantly

increased: responses to angiotensin II-val^5-amide and to vasopressin were, however, unaffected.

DISCUSSION

Female Wistar rats housed in a sound-proofed room (SPR) developed hypertension within 4 weeks; the development of this hypertension was prevented by relay of stock rat room (NRR) noises at unaltered intensity into the SPR. Hence it was the *withdrawal* of all extraneous noises that had generated the hypertension.

Hypertension generated by sound-withdrawal developed to a maximum at 4 weeks (acute phase) and stabilized at a level slightly lower than the maximum after 6 weeks (chronic phase); systemic arterioles showed thickening of the media and reduplication of the elastic lamina by the 12th week. Evidence of complex metabolic changes was found during the acute phase of the hypertension, since both organ to body weight ratios and cardiovascular responses to noradrenaline and to tyramine were significantly raised. These changes were not, however, evident in the chronic phase of the disease.

The transient changes in organ to body ratios observed during the development of sound-withdrawal hypertension contrast with those reported for renovascular hypertensive rats. In renovascular hypertension, organ to body weight ratios increase sigmoidally with time and remain elevated as the hypertension develops and stabilizes (Fregly, 1962).

The transient nature of the increased cardiovascular effects of noradrenaline and of tyramine observed during the development of sound-withdrawal hypertension is also in sharp contrast to the permanent increases in the cardiovascular responses to pressor agents characteristic of various other forms of experimental hypertension. Persistently increased pressor effects of noradrenaline, tyramine, angiotensin and vasopressin have been demonstrated in rats made hypertensive with DOCA and salt (Baum & Shropshire, 1967; Beilin & Wade, 1970; Finch, 1971). It is probable that the short duration of the treatment with DOCA and salt used in the present investigation is responsible for the failure to confirm the previous well established observations. Finch (1971) had demonstrated increased pressor effects of noradrenaline and tyramine in rats with chronic renal hypertension and Page, Kaneko & McCubbin (1966) observed increased responses of the arterial pressure to angiotensin II in dogs with renal hypertension. Nicholas (1971) contrasted the relative intensities of changes in reactivity to pressor agents in rats with renal and with DOCA and salt hypertensions. Rats with genetic hypertension also exhibit augmented pressor responses to noradrenaline (Laverty, 1961) and to angiotensin (McGregor & Smirk, 1968).

Thus, sound-withdrawal hypertension differs from other forms of experimental hypertension in genesis, and in the very transient nature of the changes in cardiovascular reactivity and in the ratio of organ to body weight found 3–5 weeks after entry to the sound-proofed room. It is possible, therefore, that further study of this new form of experimental hypertension may contribute to the elucidation of the etiology of essential hypertension.

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REFERENCES

BAUM, T. & SHROPSHIRE, A. T. (1967). *Am. J. Physiol.*, **213**, 499–503.

BEILIN, L. J. & WADE, D. N. (1970). *Nature, Lond.*, **227**, 1141–1142.

FARRIS, E. H., YEAKEL, E. H. & MEDOFF, H. S. (1945). *Am. J. Physiol.*, **144**, 331–333.

FINCH, L. (1971). *Br. J. Pharmac.*, **42**, 56–65.

FREGLY, M. J. (1962). *Am. J. Physiol.*, **202**, 967–970.

HUDAK, W. J. & BUCKLEY, J. P. (1961). *J. pharm. Sci.*, **50**, 263–264.

LAVERTY, R. (1961). *Increased vascular reactivity in rats with genetic hypertension. Proc. Univ. Otago Med. Sch.*, **39**, 23–24.

MCGREGOR, D. D. & SMIRK, F. H. (1968). *Am. J. Physiol.*, **214**, 1429–1433.

MEDOFF, H. S. & BONGIOVANNI, A. M. (1945). *Ibid.*, **143**, 300–305.

NICHOLAS, T. E. (1971). *Br. J. Pharmac.*, **42**, 179–192.

ORNSTEIN, L. (1964). *Disc electrophoresis. I. Background and Theory. Ann. N.Y. Acad. Sci.*, **121**, 321–349.

PAGE, I. H., KANEKO, Y. & MCCUBBIN, J. W. (1966). *Circulation Res.*, **18**, 379–387.

ROSECRANS, J. A., WATZMAN, N. & BUCKLEY, J. P. (1966). *Biochem. Pharmac.*, **15**, 1707–1718.

SMIRK, F. H. (1949). *Br. med. J.*, **1**, 791–799.

VERHOEFF, F. H. (1908). *J. Am. med. Ass.*, **50**, 876.