THE INFLUENCE OF ENVIRONMENTAL CHANGES ON THE CARDIOTOXICITY OF ISOPRENALINE IN RATS

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Received May 25, 1962

The influence of environment on the cardiotoxicity of isoprenaline has been examined in rats. Animals kept for 3 months in individual cages exhibited an increased sensitivity compared to their community caged controls when either mortality or the severity of the heart lesion was considered. Animals exposed to cold for one week showed a 1,000–10,000 times increased sensitivity to isoprenaline compared to their controls. A probable mechanism of sensitisation is discussed.

It has been shown by Chappel, Rona, Balazs, and Gaudry (1959) that the infarct-like myocardial necrosis produced by isoprenaline (1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride) is more severe than that produced by other catecholamines. Several factors that influence the cardiotoxicity of isoprenaline have been investigated in laboratory animals. A relation between body weight, thyroid function and the severity of isoprenaline-induced myocardial necrosis in rats has been demonstrated by Rona, Chappel, Balazs, and Gaudry (1958) and Chappel, Rona, and Gaudry (1959a). The cardiotoxicity of this compound is aggravated by mineralocorticoids (Chappel, Rona and Gaudry, 1959b), low potassium diet (Rona, Chappel and Gaudry, 1961) and excess body fat (Balazs, Sahasrabudhe, and Grice, 1962).

In the course of investigations on the influence of various diets on the cardiotoxicity of isoprenaline in rats, it appeared that those animals which were housed in individual cages showed increased sensitivity when compared with community caged animals. It is known that long-term individual housing of laboratory animals has an effect on behaviour and physiological responses to stress (Stern, Winokur, Eisenstein, Taylor, and Sly, 1960) but the effects of such housing on toxicological responses have not been explored.

The first part of this study was undertaken to compare the cardiotoxicity of isoprenaline between individually and group-caged rats.

Experimental

Rats of the Wistar strain bred and raised in this laboratory were kept in an air-conditioned room at a temperature of 24 $\pm$ 1° and a relative humidity of 45 $\pm$ 5 per cent. They had a commercial diet,* and water *ad libitum*, and were weighed weekly but were not handled otherwise.

In one experiment 80 female rats, 5–6 weeks of age and weighing 50–60 g. were divided into two comparable groups. Forty rats were placed in large community cages (50 $\times$ 50 $\times$ 25 cm.), each housing 10 rats, and

* Fox Chow, Toronto Elevators Ltd., Toronto, Canada.

forty were in individual cages (28 × 20 × 20 cm.) with metal sides and screened rear wall, floor and door. The rats were housed for 3 months in the manner described. At the end of 3 months, housing was exchanged between 10 community caged animals and 10 individually caged animals for 1 week. This was done to determine whether a short term exchange in the housing of the animals would modify the cardiotoxicity of isoprenaline. The mean terminal body weights of the rats were 199 ± 3 g. for the individually caged and 199 ± 4 g. for the community caged groups.

In a second experiment 100 rats 5–6 weeks of age and weighing 50–70 g. were divided into two groups of 25 females and 25 males each. One group was placed in community cages, the other in inidividual cages as described above for 3 months. At the end of the 3 months housing the mean body weights for the individual and community groups respectively were as follows: females, 188 ± 3 g. and 199 ± 3 g.; males, 336 ± 6 g. and 322 ± 7 g.

Injections of isoprenaline were given after the 3-month preparatory period. A single injection of an aqueous solution of isoprenaline hydrochloride was given subcutaneously at two dose levels in the first experiment. In the second experiment four dose levels were used and those animals that survived for 24 hr. were given a second injection. The doses were the same as that given for the first injection. The number of rats injected and the doses in mg./kg. are shown in Tables I and II. Control rats were injected with saline. Mortality was recorded up to 24 hr. The myocardial lesions in the survivors and in those dying after the second injection were graded as described by Rona and others (1959b). The grading was done under single blind conditions. Differential white cell counts and the weights of adrenals were taken. Adrenal ascorbic acid content was determined using the method of Bessey (1938) on the saline injected controls.

Results

The manner of housing had a pronounced effect on the rat's behaviour. The individually caged animals in this experiment were excitable and intractable whereas the community caged rats were docile and easily handled. Such disturbances in behaviour of experimental animals kept in solitary confinement have been observed by Barnes (1960).

The results of the isoprenaline treatment are summarised in Tables I and II. In the first experiment (Table I) a single dose of either 100 or 50 mg./kg. of isoprenaline caused severe dyspnoea, prostration and convulsions in the individually caged rats. Nine out of ten rats in this group died at both dose levels. A temporary dyspnoea without mortality was observed in the animals kept in community cages and receiving 100 or 50 mg./kg. doses. The ten community caged rats, transferred into individual cages for a one-week period before isoprenaline administration, were not killed by a 100 mg./kg. dose. However, individually caged animals placed in community cages for 1 week died from this dose.

In the second experiment (Table II) the mortality of the females, housed individually, was comparable to that obtained in the first experiment.

Furthermore, the pathological findings indicated an increased sensitivity of the isolated females. Twenty mg./kg. of isoprenaline induced more severe cardiac lesion in these rats (grade 3·5) than did a 200 mg./kg. dose in community caged females (grade 1·5). The cardiotoxicity of isoprenaline in males was also influenced by isolation. This was shown by mortality at the 100 and 200 mg./kg. doses (5/5 vs. 1/5) and also by the grade of myocardial damage at the 20 and 50 mg./kg. dose levels (3·5 vs. 1; 4 vs. 2 respectively). However, the community caged male rats were more sensitive to the cardiotoxic effect of the amine than were the community caged females. This is evident when both mortality and the severity of the myocardial lesion at the 100 and 200 mg./kg. dose levels are considered.

TABLE I

COMPARISON OF THE EFFECT OF ISOPRENALINE ON MORTALITY OF INDIVIDUALLY AND COMMUNITY-CAGED FEMALE RATS

Experiment I

Group	Dose mg./kg.	Mortality
Individual*	100	9/10
	50	9/10
	0	0/10
Community*	100	0/10
	50	0/10
	0	0/10
Individual* Exchanged‡ to Community	100	10/10
Community* Exchanged‡ to Individual	100	0/10

* Caged for 3 months. ‡ Exchanged for 1 week.

Differential white cell counts and adrenal ascorbic acid content were the same in the individual and community caged groups. There was also no significant difference between the adrenal weights of the individually and community caged rats. The hearts in the saline treated groups appeared normal on gross and histologic examination. No evidence of intercurrent disease was observed in any groups of this study.

The results of this study indicate that rats housed for 3 months in individual cages develop an increased sensitivity to the cardiotoxic effect of isoprenaline. A single dose of 50 mg./kg., which caused mortality in the individually caged female rats represents approximately 6 per cent of the LD50 of 815 $\pm$ 70 mg./kg. as determined in this laboratory for community caged 200 g. female rats (Balazs, Sahasrabudhe and Grice, 1962). The community caged males showed an increased sensitivity as compared with the community caged females. It is known that a positive relationship exists between body weight and isoprenaline toxicity (Rona and others, 1958) and the increased susceptibility to this compound in the heavier males may be explained on this basis. Because of the effect of this weight factor the sensitivity difference between individually and

community caged rats was not as pronounced for the males as for the females in this study.

The sensitivity to the cardiotoxic effect of isoprenaline was not altered by a period of 1 week of exchanged housing, thus the underlying mechanism of the sensitisation is not of an acute character.

TABLE II

COMPARISON OF CARDIOTOXICITY OF ISOPRENALINE IN INDIVIDUALLY AND COMMUNITY-CAGED RATS

Experiment II

Group	Dose* mg./kg.	Mortality	Average grade of myocardial lesions
Females—			
Individual	200	5/5	—
Community	200	0/5	1·5
Individual	100	4/5	3·0
Community	100	0/5	1·5
Individual	50	4/5	3·0
Community	50	0/5	1·5
Individual	20	0/5	3·5
Community	20	0/5	1·0
Individual	0	0/5	0
Community	0	0/5	0
Males—			
Individual	200	5/5	—
Community	200	1/5	4·0
Individual	100	5/5	—
Community	100	1/5	4·0
Individual	50	1/5	4·0
Community	50	0/5	2·0
Individual	20	1/5	3·5
Community	20	0/5	1·0
Individual	0	0/5	0
Community	0	0/5	0

* 24 hr. survivors received a second similar dose.

EXPOSURE TO COLD STRESS

Since a minor environmental change was found to have an effect on the cardiotoxicity of isoprenaline further experiments were made to examine the effect of a more drastic change of the environment. Exposure of rats to cold was chosen for this purpose. The physiological changes accompanying cold exposure in rats have been reviewed by Smith and Hoijer (1962). It was thought that if a major environmental change having a known physiological effect influenced more profoundly the cardiotoxicity of this compound, it might shed some light on the mechanism of sensitivity due to such changes.

EXPERIMENTAL

Seventy-two male rats of the Wistar strain, bred and raised in our laboratory, were divided into two groups of 36. The rats were assigned to each group so that the weights were comparable; cold exposed:

360 ± 17 g.; control: 378 ± 20 g. The first group were placed in individual cages in a cold room having a mean temperature of 4 ± 1°. The second group were kept at room temperature (24 ± 1°) also in individual cages. All rats were fed water and food *ad libitum*.

After seven days the rats in each environment were randomly subdivided into 6 groups of 6 and injected with various doses of isoprenaline subcutaneously. The cold exposed rats were taken from the cold room, injected immediately and were then placed in individual cages in the same room as the controls. Doses and mortality are presented in Table III.

In the second experiment 48 female rats weighing 260–280 g. were divided into two groups of 24 each. The rest of the experiment was conducted as described above, except that the rats from both environments were divided into 4 groups of 6. Doses and mortality are presented in Table III.

Animals were necropsied and the adrenal weights were recorded. Hearts from both groups were examined histologically.

Results and Discussion

The results of the experiments are summarised in Table III. Severe dyspnoea was observed in both groups, followed by a shock-like stage. It is apparent that a remarkable sensitivity to isoprenaline is induced

TABLE III

COMPARISON OF THE EFFECT OF ISOPRENALINE ON MORTALITY IN RATS EXPOSED FOR ONE WEEK TO COLD AND ROOM TEMPERATURE

Group	Dose mg./kg.	Mortality
Control males	220	2/6
	180	1/6
	160	2/6
	140	3/6
	120	1/6
	100	0/6
Cold exposed males	20	6/6
	10	5/6
	5	6/6
	1	6/6
	0·75	5/6
	0·25	4/6
Control females	800	2/6
	700	2/6
	600	3/6
	500	1/6
Cold exposed females	10	6/6
	5	6/6
	0·1	4/6
	0·01	0/6

in rats that have been exposed to a cold environment for 1 week. In the male rats the difference in sensitivity compared to control rats appears to be about 1000. The cold exposed female rats in the second experiment appear to be even more sensitive to the drug; the difference being about 10,000.

The adrenal weights of both male and female rats exposed to cold increased as compared with those of the control rats. However, this was significant statistically only in the females ($P < 0.05$). It is interesting to note that the cold exposed females developed a proportionately higher sensitivity when compared with their controls than did the cold exposed males. However, the control males were more sensitive to the amine than the control females because of their heavier body weight.

Histological examination of the heart revealed separation of muscle fibre with oedema and haemorrhage. No evidence of intercurrent disease was observed in any groups of this study.

That thyroid and adrenal weights increase during cold exposure is well documented (Smith and Hoijer, 1962). Chappel and others (1959a, 1959b) have shown that thyroid hormone and mineralocorticoids increase the cardiotoxicity of isoprenaline in rats. Raab and others (1960), demonstrated, in rats exposed to certain stressful situations, that these hormones sensitised the myocardium to the cardiotoxic properties of catecholamines. There is thus reason to believe that these hormones played a role in the sensitisation of the myocardium to isoprenaline in our rats.

A drastic environmental change, like cold exposure, which is known to alter homeostasis, had a potent effect in sensitising rats to isoprenaline while a minor environmental change, like long-term isolation, induced a minor increase in sensitivity to the amine. It is possible that both environmental changes produced the increased sensitivity in rats by a common mechanism of action.

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