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# Endogenous Levels of Catecholamines in the Rat Myocardium Following Exposure to Stress

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BASSETT, J. R. AND K. D. CAIRNCROSS. *Endogenous levels of catecholamines in the rat myocardium following exposure to stress.* PHARMAC. BIOCHEM. BEHAV. 4(1) 35–38, 1976. – Following exposure of male rats to irregular signalled footshock from which they could escape, the endogenous level of norepinephrine in the myocardium was significantly reduced. In atria the depletion of endogenous norepinephrine was significant following 1 day of stress. This significant depletion was maintained following 4 days of stress but began to return towards control levels by Day 10. A similar, but less pronounced pattern was seen with the ventricles. Little if any epinephrine was detected in both control and stress atria and ventricles. From the results presented it is postulated that irregular signalled footshock results in an inhibition of neuronal uptake.

Myocardium Catecholamines Stress

PREVIOUS work from this laboratory has shown that exposure of male rats to irregular footshock will induce an enhanced myocardial sensitivity to norepinephrine (NE) and epinephrine (Epi) [3]. While the precise etiology of the enhanced sensitivity has not been elucidated a number of possible mechanisms were proposed. It was suggested that the enhanced myocardial sensitivity of catecholamines could result from three mechanisms, acting singly or in combination. These relate to the following: (1) Inhibition of the catecholamine catabolizing enzymes and in particular catechol-O-methyl transferase (COMT), (2) Inhibition of the active uptake mechanisms for catecholamines into either neuronal or extraneuronal storage sites, (3) A direct change in the sensitivity of the effector cell itself. Elevation of plasma corticosterone is reported to be implicated with each of these mechanisms [2] and extreme elevation of plasma glucocorticoids have been demonstrated as a sequel to this form of stress [2,3].

As a consequence of these observations and in view of previous work described, it was decided to examine endogenous NE and Epi levels in rat hearts following exposure to stress, as changes in levels of the endogenous catecholamines could indicate which of the mechanisms mentioned above are involved in the sensitivity change.

## METHOD

### *Animals*

Male CSF rats 87–93 days old were used in this experiment. The animals were housed in groups of 3 under conditions of constant temperature and humidity (21 ± 0.5° C, 46 percent humidity) and subjected to a 12 hr night-day routine (light 8 a.m. – 8 p.m.) beginning at least 14 days prior to commencement of experimentation.

### *Apparatus and Procedure*

The apparatus and stress parameters for the irregular, signalled footshock from which the animal could escape (Irreg.-sig. Escape) were the same as that described by Bassett *et al.* [2].

Naive animals were placed in automated 1-way avoidance boxes (Lafayette Model No. 85200) described in detail by Bassett *et al.* [2]. An escape platform was made available to the animal by an automated movable partition. A light conditioned stimulus (CS) of 2W was located on the wall of the grid chamber opposite to the escape platform. The unconditioned stimulus (UCS) was delivered by a generator-scrambler through the grids as a 2mA, 50 pulses/sec square wave. Each rat was placed on the escape platform at the commencement of the treatment session. Treatment consisted of 7CS-UCS exposures randomly placed in the 35 min session. On each trial the CS onset 4 sec before the animal was pushed by the movable partition from the platform onto the grid which was simultaneous with the onset of the UCS. At this the movable partition immediately retracted and the animal was able to jump from the grid to the re-exposed platform with a minimum latency of 0.3 sec. The UCS was terminated by the return of the animal to the platform. Such a stress procedure where the CS was associated with an irregular UCS has been shown to induce an extremely high plasma level of

corticosterone independent of shock intensity or duration [2]. While there is a significant improvement in escape performance over the first 3 days of stress no further significant alteration occurs after this period.

The stress procedure was repeated daily for periods of 1, 4 and 10 days between the hours 9 a.m. – noon. Animals were sacrificed by cervical dislocation immediately following completion of the last stress period. The heart was removed and the atria and ventricles dissected free from arterial and venous segments. The atria and ventricles were weighed separately, then rapidly frozen using liquid nitrogen and stored at −20°C. Control animals were killed over a similar time period and then atria and ventricles prepared in an identical manner. Determinations of endogenous Epi and NE levels in atrial and ventricular tissue were carried out using modified versions of the aluminium oxide chromatographic method of Anton and Sayre [1] for extraction, and the fluorimetric assay of Haggendal [9] for analysis. The calculation of NE and Epi levels in the presence of each other was accomplished by the solving of simultaneous equations as detailed by Haggendal [9], fluorescence values being recorded at two different wavelengths.

## RESULTS

The endogenous NE levels in both atrial and ventricular tissue from control and stressed animals are shown in Fig. 1. A one way analysis of variance on the control levels of endogenous NE showed no significant variation over the course of the experiment in either atria or ventricles. In the absence of a significant variation the control values were pooled for both these tissues and the pooled values are represented as the Day 0 points of Fig. 1. Endogenous NE levels from stressed animals were compared statistically against the pooled control values using an unpaired $t$ test.

In atria a significant depletion in endogenous NE occurred following exposure to 1 day of stress ($p<0.01$, $df = 18$). This significant depletion was maintained following 4 days of stress ($p<0.005$, $df = 18$) but began to return towards control levels by Day 10 ($p>0.10$, $df = 17$). A similar, but not as pronounced, pattern was seen with the ventricles. A small, non-significant, depletion of endogenous NE occurred following 1 day of stress ($p>0.05$, $df = 16$), being maintained for 4 days ($p>0.30$, $df = 18$) and returning to control values by Day 10 ($p>0.80$, $df = 16$).

In both control and stressed atria and ventricles the level of endogenous Epi was generally too low for analysis. However, in recovery experiments 0.017 μg of Epi produced measurable fluorescence and this quantity when expressed as μg Epi/g tissue is below that reported for the endogenous levels of Epi in cardiac tissue [8,17]. Trace amounts of Epi (<0.013 μg/gm tissue) were detected in the ventricles of some stressed animals, especially those exposed to 10 days stress. No detectable Epi was seen in control animals or atria of stressed animals.

## DISCUSSION

Activation of the adrenal medulla in response to stress results in an elevated plasma level of Epi [14]. Much of this circulating Epi is rendered inactive by rapid uptake into peripheral stores, especially those within the heart [23]. It follows therefore that in the absence of any alteration in uptake or catabolism of the catecholamine, release of Epi from the adrenal medulla in response to stress would be expected to increase the endogenous level of the hormone

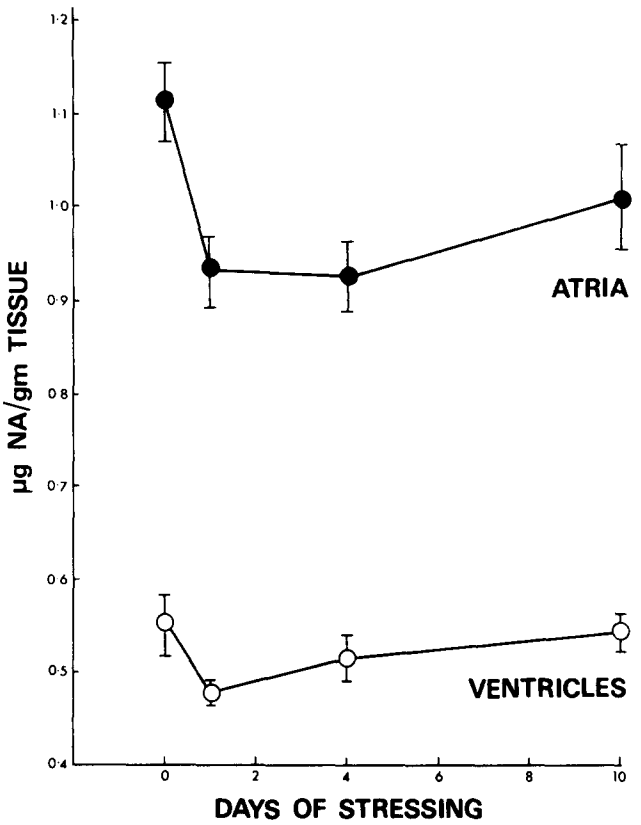


FIG. 1. Endogenous levels of norepinephrine in rat atria and ventricles of control animals (0 days), and animals exposed to 1, 4 and 10 days of irregular-signalled escape stress. Each period represents the mean value, horizontal bars designate ± S.E.

Number of analyses/group.

| | Control | 1 day | 4 days | 10 days |
|---|---|---|---|---|
| Atria | 11 | 9 | 9 | 8 |
| Ventricles | 10 | 8 | 9 | 8 |

in the myocardium. However, such a situation would not be expected with regard to endogenous NE levels, where exposure of the animal to stress would be anticipated to produce little change. The basis for this comment relates to the fact that although increased sympathetic nerve activity occurs in stress situations [14] it is not easy to deplete the stores of NE by nerve stimulation. Re-uptake of the released NE by the nerve terminals, and the ability of the terminals to synthesize the transmitter, are normally capable of maintaining the stores. Even relatively prolonged stimulation, leading to a marked increase in the output of the transmitter, is accompanied at most by a very small reduction in the NE content of the organ [5,22].

If a direct sensitization of the effector cell or an inhibition of the catabolizing enzyme COMT were responsible for the enhanced myocardial sensitivity to catecholamines reported by Bassett and Cairncross [3], then exposure to the same stressor should result in an elevation

in the endogenous levels of Epi in the myocardium when compared to non-stressed controls. The elevation in Epi levels should be accompanied by little or no change in the endogenous level of NE or, if COMT is inhibited, a possible elevation in the level of NE may be observed, more NE being available for uptake into storage sites.

In the experiments described there occurred a depletion of endogenous NE and apparent absence of Epi in the myocardium following exposure to stress. Such findings would suggest that the enhanced myocardial sensitivity previously described [3] cannot be attributed to either a direct sensitization of the effected cell or to inhibition of COMT. The results, however, are consistent with the premise that an inhibition of catecholamine uptake into neuronal and/or extra neuronal stores has occurred. Such a depletion of myocardial NE has been described in rats following prolonged intermittent foot-shock and isolation. The same study reported a small rise in cardiac Epi, particularly in the ventricle [17]. Depletion of cardiac NE and epinephrine has been described in rats following systemic administration of corticoids [8]. This was attributed to excitation of the adrenal-sympathetic axis. Not all studies associated with circumstances in which elevation of circulating corticosteroids could be predicted have reported depletion of catecholamines; this applies particularly to the central nervous system. Foot-shock in rats was without effect on the disposition of catecholamines localised in various intraneuronal storage forms in the brain stem [21]. This observation could be interpreted as a failure of corticosteroids to affect neuronal uptake, or as a reduced access of corticosteroids to catecholamine storage sites within the central nervous system.

The supposition that enhanced myocardial sensitivity to catecholamines relates to extreme glucocorticoid elevation receives support from several studies using exogenous steroids. These demonstrate an inhibition of extraneuronal uptake ($Uptake_2$) in the presence of corticosteroids [10, 12, 16]. However, a similar inhibition of neuronal uptake ($Uptake_1$) has not been demonstrated. In this regard it is of interest that in this experiment the depletion of endogenous NE is more pronounced in the atria rather than the ventricles, suggesting that neuronal uptake as well as extraneuronal uptake may be inhibited, for the following reasons. In the rat heart, sympathetic nerve fibres innervate both atria and ventricles, the ventricles having a moderately dense adrenergic innervation [4]. However, there is a tendency for nerve endings to accumulate around the sinoatrial node and atrioventricular node resulting in a greater sympathetic innervation to the atria compared with the ventricles [6,24]. Further, in the isolated rat heart up to 95 percent of the uptake of exogenous NE is restricted to sympathetic nerve endings, nevertheless uptake of catecholamines does take place into cardiac muscle cells especially at high concentrations [11]. The relative density of $Uptake_1$ and $Uptake_2$ sites will vary according to the density of the sympathetic innervation [11]. It is reasonable to suggest therefore, that the $Uptake_2$ mechanism might have a more important physiological role in ventricle than in atrial tissue. This assumption would explain the observation of Ordy *et al.* [17], which is tacitly supported by the results reported in this paper, that a greater accumulation of Epi occurs in the ventricles compared to the atria following prolonged exposure to stress. $Uptake_1$ shows a greater preference for NE than Epi whereas $Uptake_2$ has a higher affinity for Epi than NE. Circulating Epi would be rapidly removed by $Uptake_2$ sites for which it has a high affinity. After intravenous injection of small doses of $^3$H-epinephrine only about 35 percent of the injected dose appears to be taken up and retained by the neuronal mechanism [13]. If there are a greater number of extraneuronal sites within the ventricle compared with the atrium then, provided both regions are exposed equally to the elevated circulating Epi levels, the uptake of Epi should be greatest in the ventricles. Under the circumstances where the proportion of neuronal sites is greatest in the atria, inhibition of neuronal uptake in stress situations should result in a more pronounced depletion of endogenous NE in the atrial tissue.

Bassett and Cairncross [3] found that the enhanced myocardial sensitivity to catecholamines observed after exposure to stress persisted unchanged over the duration of the 28 day stress period. However, the endogenous NE levels tend to recover to pre-stress levels within 10 days. It is reasonable to suppose that such a recovery reflects an enhanced NE synthesis rate. During periods of increased catecholamine utilization resulting from direct nerve stimulation the synthesis of catecholamines from tyrosine is rapidly increased [7, 18, 19, 20]. This increased synthesis has been postulated to be a consequence of decreased end-product inhibition of tyrosine hydroxylase [15].

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