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Myocardial Sensitivity to Catecholamines Following Exposure of Rats to Irregular, Signalled Footshock[1]

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BASSETT, J. R. AND K. D. CAIRNCROSS. *Myocardial sensitivity to catecholamines following exposure of rats to irregular, signalled footshock.* PHARMAC. BIOCHEM. BEHAV. 4(1) 27–33, 1976. – Emotional stress is associated with an increased activity of both the pituitary-adrenal cortical system and the sympathetic-adrenal medullary systems resulting in raised plasma levels of glucocorticoids and catecholamines. There is evidence to suggest that prolonged stress induced adrenergic hyperactivity initiates myocardial pathogenesis and that this may relate to a corticosteroid catecholamine interaction. In the present study driven atrial strips removed from stressed male CSF rats were found to exhibit an enhanced sensitivity to both norepinephrine and epinephrine. These animals had previously been subjected to irregular foot shock associated with a warning signal; a situation producing a high plasma steroid level. The enhanced myocardial sensitivity to both catecholamines was observed in naive animals subjected to a single stress period, and persisted unchanged in animals stressed daily over a 28 day period. The hypersensitivity of the myocardium observed immediately after stress was maintained for at least 24 hr, whereas the circulating steroid level had returned to control values within 3 hr. In animals subjected to regular stress without a warning signal, a situation producing a much lower steroid level, no enhanced myocardial sensitivity was observed. While the aetiology of the phenomenon of enhanced myocardial sensitivity to catecholamines is not entirely understood, the evidence presented suggests that it may be related to the extreme elevation of circulating glucocorticoids. The sensitivity of the vas deferens however, was unaltered even though the animals were subjected to the stressor producing a high plasma steroid level. This apparent specificity of the stress induced sensitivity change is discussed on the basis of receptor differences.

Myocardium Catecholamines Stress Plasma steroids

IN a previous paper, Bassett and Cairncross described the morphological changes produced in the rat heart following exposure of the animals to prolonged unpredictable stress [4]. It was suggested that the myocardial changes observed might relate to the extreme glucocorticoid elevation induced in the rat following exposure to this form of stressor [3]. The basis of this suggestion relates to a possible potentiation of catecholamine action by glucocorticoids released in a stress situation. The concept of such an interaction has been discussed by other workers, and has been suggested as an initiating factor in the pathogenesis of cardiovascular disease [44]. However, the etiology of such an interaction has not been elucidated.

A review of the literature suggests that the glucocorticoids themselves do not appear to exert a positive inotropic or chronotropic effect on the myocardium [1], although this point is disputed [12, 41, 49]. However glucocorticoids do potentiate some effects of the sympathomimetic amines on the cardiovascular system. Potentiation by corticosteroids of the pressor effects of catecholamines, and catecholamine induced vasoconstriction in certain vascular beds, has been observed by many investigators [45]. The vasoconstrictor response to epinephrine is enhanced by hydrocortisone in the perfused hindquarters of the cat, and in the perfused cephalic saphenous venous systems of the dog. However, hydrocortisone is reported not to increase the constrictor response to norepinephrine or sympathetic nerve stimulation in these preparations, or in the perfused vascular beds of the cat intestine or kidney [24, 25, 51]. The dilator response to isoprenaline in these vascular beds also remains unaltered by hydrocortisone [51]. Similarly, Hess and Shanfield [18], using the open chest rat preparation, were unable to demonstrate any potentiation of the effects of epinephrine on blood pressure, or cardiac inotropic and chronotropic responses following cortisol or corticosterone administration. Conversely, in vitro studies using the isolated aortic strip have reported that hydrocortisone potentiates the response to both epinephrine and norepinephrine.

It would appear therefore, that despite considerable investigation no clear picture has emerged regarding glucocorticoid enhancement of catecholamine action on the

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cardiovascular system. However, the studies discussed have utilised exogenous steroid, and it would be more relevant to study the postulated steroid-catecholamine interaction in vivo, where endogenous levels of glucocorticoid would be elevated. Indeed studies examining the metabolic actions of norepinephrine in the presence of cold stress have shown such actions to be enhanced [33,34]. Previous studies from this laboratory have indicated that two distinct levels of glucocorticoid elevation can be achieved in the rat using different stress regimens. Thus, irregular signalled footshock with the possibility of escape produces a plasma glucocorticoid level of 85–90 μg per 100 ml plasma, i.e. extreme elevation, and regular, unsignalled footshock with no escape possibility produces an intermediate steroid elevation of 45–50 μg per 100 ml plasma [3]. These observations offered the opportunity of examining the actions of epinephrine and norepinephrine on the myocardium with two different plasma levels of endogenously released glucocorticoid. It was decided also, to examine the adrenergic sensitivity of the vas deferens in the hope of determining the specificity of the glucocorticoid-catecholamine interaction.

METHOD

Animals

Male CSF rats 87–93 days old were used in all experiments. 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 and continuing until its conclusion. Food and water were provided ad lib. Both control and stressed rats were housed under identical conditions.

Apparatus and Procedure

The apparatus and stress parameters for the regular, unsignalled footshock (Reg.-unsig.) and the irregular, signalled escape footshock (Irreg.-sig. Escape) were the same as that described by Bassett *et al.* [3]. In the case of the Reg.-unsig. group naive animals were placed in a white Plexiglas box of internal dimensions 34 × 23 × 33 cm high with a grid floor of stainless steel 0.65 cm dia. rods set at 1.9 cm centres. The unconditioned stimulus (UCS) was 2 sec of scrambled footshock repeated every 88 sec and delivered through the grid floor as a 2 mA, 50 pulses/sec DC square wave. For the Irreg.-sig. Escape group the animals were placed in automated 1-way avoidance boxes (Lafayette Model No. 85200) described in detail by Bassett *et al.* [3]. 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 UCS was delivered by a generator-scrambler through the grids as a 2 mA, 50 pulses/sec square wave. Each rat was placed on the escape platform at the commencement of the treatment 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 subject 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. Both Reg.-unsig. and Irreg.-sig. Escape stress procedures were carried out between the hours 9 a.m. –12 noon. In both stress procedures the duration of a stress session was 35 minutes.

At a set time after the completion of the last stress episode the animals were sacrificed by cervical dislocation and exsanguinated. The blood was collected in heparinized tubes and centrifuged in order to obtain cell free plasma which was then frozen. Corticosterone levels in plasma were determined subsequently by the fluorimetric method of Mattingley [37], which is specific for free 11-hydroxy-corticosteroids. The number of days of stress and the time of death (in hours) after completion of the last stress period are shown in the results section.

Isolated tissues. Atrial strips or vas deferens were obtained from both stressed and control animals. The preparations were suspended in Krebs-Henseleit solution gassed with 5 percent carbon dioxide in oxygen. The Krebs solution had the following composition: NaCl, 118 mM; KCl, 4.7 mM; $NaHCO_3$, 25.0 mM; $MgSO_4$, 0.45 mM; KH_2PO_4, 1.03 mM; $CaCl_2$, 2.5 mM; and D (+) glucose, 11.1 mM. Atrial preparations were maintained at 32.5°C and vas deferens at 32.0°C. Both tissues were allowed to equilibrate for 30 minutes after setting up before commencement of experiment.

Electrically driven atrial strip. The technique was similar to that described by Blinks [6]. A triangular segment with a base of 3 mm and a height of 7 mm was cut from the wall of the left atrium. The base of this segment was clamped in an electrode assembly and its apex connected to an isotonic strain gauge transducer. The atrial segment was stimulated with pulses of 1 msec duration, at a frequency of 1 Hz delivered through a punctate platinum electrode. The voltage used was just sufficient to elicit contraction (<3 volts). Contraction amplitude was recorded using a Cardiotrace recorder.

Vas deferens. The vas deferens was removed and cleaned of connective tissue. Approximately 5 cm of tissue was then suspended in an organ bath. Changes in length of the preparation were measured isotonically at a tension of 300 mg using a strain gauge transducer, and were recorded on a Rikadenki pen recorder.

Log dose-response curves for both norepinephrine and epinephrine were obtained on both tissues. In the case of the atrial strip, the curves were obtained by the cumulative method, each curve being triplicated. With the vas deferens doses were added singly and the log dose-response curve obtained using a 4 × 3 assay. For both tissues responses were expressed as a percentage of the maximal response determined for each curve. Regression lines were fitted to the linear portions of the curves by the method of least squares. Each line was tested statistically for linearity and the ED_{50} for the catecholamines determined using the regression coefficients. ED_{50} was used as a measure of sensitivity in this series of experiments.

In order to minimise variations in the sensitivity to catecholamines that occur due to slight changes in the temperature and composition of the Krebs solution, home conditions etc. between experiments, each stressed group was tested against its own control group. Control animals were randomly selected before the beginning of the experiment. As far as possible conditions were kept unchanged throughout an experiment. Control and stressed ED_{50}'s were measured simultaneously.

TABLE 1

MYOCARDIAL SENSITIVITY TO CATECHOLAMINES MEASURED 24 HOURS AFTER COMPLETION OF STRESS, IN RATS SUBJECTED TO IRREGULAR-SIGNALLED ESCAPE STRESS

	Mean ED_{50} ($\times 10^{-8}$ M) ± SE		
	Control	Stressed	*t* Test *p*
Epinephrine	15.1 ± 2.6	5.7 ± 0.5	<0.02
Norepinephrine	18.5 ± 2.5	10.4 ± 0.8	<0.02
11-OHCS (μg/100 ml)	4.6 ± 0.9	8.8 ± 0.9	

$p<0.05$ is significant

RESULTS

Myocardial Sensitivity Following Irregular-Signalled Escape Stress

The myocardial sensitivity to both norepinephrine and epinephrine following stress associated with a high circulating level of corticosterone (90 μg/100 ml plasma) is shown in Table 1. Stressed animals were killed in groups of 2 at 3 day intervals 24 hr after the completion of the last stress period, i.e. rats were killed after 1, 4, 7, 10 etc. days of stress up to a total of 28 days. Control animals were killed over a similar time period.

No correlation was found between myocardial sensitivity and days of stress for both catecholamines in either stressed or control groups. The various correlation coefficients, calculated values of *t* and probabilities are shown in Table 2. In the absence of a significant correlation the results for individual animals were pooled in both control and stressed groups. The sensitivity of the myocardium to both epinephrine and norepinephrine was then compared between the two groups using a *t* test. The results are shown in Table 1. The ED_{50} for both epinephrine and norepinephrine was significantly lowered in the stressed animals indicating that the sensitivity of the myocardium to both catecholamines was significantly enhanced following stress. The mean plasma 11-hydroxycorticosteroid level in the stressed animals, which initially would have been approximately 90 μg/100 ml plasma [3], had returned to within normal control levels in the 24 hr period between the completion of the last stress period and the sacrificing of the animal.

In order to determine the time course for onset and decay of this change in myocardial sensitivity to catecholamines following stress, animals were subjected to 4 days of stress and sacrificed at 0, 3 and 48 hr following completion of the last stress period. The results of this study are shown in Table 3. In both the 0 hr and 3 hr groups a significant increase in myocardial sensitivity to both catecholamines was observed following stress. Forty-eight hr after the completion of the last stress episode however, no significant change in the sensitivity of either catecholamine was

TABLE 2

VARIATION IN MYOCARDIAL SENSITIVITY TO CATECHOLAMINES AS A FUNCTION OF THE NUMBER OF DAYS OF EXPOSURE TO IRREGULAR SIGNALLED ESCAPE STRESS

	Control		Stressed	
	Epinephrine	Norepinephrine	Epinephrine	Norepinephrine
r	0.062	0.345	0.224	0.101
t_{calc}	0.138	0.822	0.889	0.404
p>	0.80	0.40	0.30	0.60
n	10	10	19	19

r = correlation coefficient t_{calc} = calculated value of *t* n = number of animals/group

TABLE 3

MYOCARDIAL SENSITIVITY TO CATECHOLAMINES IN RATS SUBJECTED TO 4 DAYS OF IRREGULAR-SIGNALLED ESCAPE STRESS

	Mean ED_{50} ($\times 10^{-8}$M) ± SE		
	Control	Stressed	*t* Test *p*
0 Hours			
Adren	13.5 ± 2.0	5.5 ± 0.6	<0.01
Noradren	21.0 ± 2.6	6.2 ± 0.8	<0.005
11-OHCS (μg/100 ml)	7.9 ± 1.8	83.0 ± 6.3	
3 Hours			
Adren	19.6 ± 2.4	10.1 ± 1.6	<0.005
Noradren	24.1 ± 3.1	13.1 ± 1.6	<0.005
11-OHCS (μg/100 ml)	6.3 ± 1.3	3.0 ± 0.5	
48 Hours			
Adren	12.6 ± 1.1	12.1 ± 2.5	>0.8
Noradren	17.4 ± 3.3	14.5 ± 2.6	>0.3
11-OHCS (μg/100 ml)	11.5 ± 4.4	6.4 ± 1.1	

number of animals/group = 6 Adren = Epinephrine Noradren = Norepinephrine

observed. Immediately following the last stress episode (0 hr) the enhanced sensitivity was associated with an elevated plasma steroid level, comparable with those previously reported [3]. By 3 hr the level of circulating steroid had returned to control values.

Myocardial Sensitivity Following Regular-Unsignalled Stress

The myocardial sensitivity to norepinephrine and epinephrine in rats subjected to stress associated with an intermediate steroid response (49 μg/100 ml plasma) is shown in Table 4. Animals were subjected to 4 days of stress and killed immediately after the completion of the last stress period. No change in the myocardial sensitivity was observed following exposure to such a stress procedure. The plasma corticosteroid response was comparable with that reported for the same stressor by Bassett *et al.* [3].

Sensitivity of the Vas Deferens to Catecholamines Following Irregular-Signalled Escape Stress

Animals were stressed for 4 days and killed immediately after the completion of the last stress period. The sensitivity of the vas deferens to norepinephrine and epinephrine in stressed and control groups is shown in Table 5. No significant change in the sensitivity of the vas deferens to either catecholamine was observed. The plasma steroid level immediately following completion of the last stress episode was elevated to a level comparable with that reported previously for this stress procedure.

DISCUSSION

Exposure of rats to irregular-signalled escape stress, a stressor associated with an extreme plasma corticosteroid elevation resulted in enhanced myocardial sensitivity to both norepinephrine and epinephrine. While the results reported in this study do not allow categoric statements to be made regarding the nature of the enhanced myocardial sensitivity, several interesting points emerge. First, enhanced sensitivity could only be demonstrated in the myocardium of rats in which an extreme plasma glucocorticoid level was induced. No enhanced sensitivity was observed in rats exposed to the stressor inducing moderate steroid elevation. It would appear therefore, that enhanced myocardial sensitivity could relate to the level of circulating glucocorticoids. However, while the level of circulating glucocorticoids tends to return to normal values within 3 hr following the completion of the stress period, an observation supported by the findings of others [8, 13, 29], the enhanced sensitivity persists for at least 24 hr. Such a persistent enhanced sensitivity suggests that only one glucocorticoid surge in a 24 hr period would be sufficient to maintain the heart in a state of constant enhanced sensitivity. Further exposing the animal to repeated stress periods does not potentiate the enhanced sensitivity; the

TABLE 4

MYOCARDIAL SENSITIVITY TO CATECHOLAMINES IN RATS SUBJECTED TO 4 DAYS OF REGULAR-UNSIGNALLED STRESS

	Mean ED_{50} ($\times 10^{-8}$M) ± SE		*t* Test
	Control	Stressed	*p*
0 Hours			
Epinephrine	6.9 ± 0.5	7.3 ± 1.4	>0.70
Norepinephrine	9.2 ± 1.0	9.2 ± 1.3	>0.95
11-OHCS (μg/100 ml)	10.0 ± 2.6	49.0 ± 4.2	

number of animals/group = 6

TABLE 5

SENSITIVITY OF THE VAS DEFERENS TO CATECHOLAMINES IN RATS SUBJECTED TO 4 DAYS OF IRREGULAR-SIGNALLED ESCAPE STRESS

	Mean ED_{50} ($\times 10^{-8}$M) ± SE		*t* Test
	Control	Stressed	*p*
0 Hours			
Epinephrine	11.1 ± 0.7	12.8 ± 2.5	>0.50
Norepinephrine	36.2 ± 1.2	40.3 ± 2.6	>0.20
11-OHCS (μg/100 ml)	14.2 ± 1.4	101 ± 12.6	

number of animals/group = 6

response is observed in naive animals, and persists unchanged in animals stressed daily for a 28 day period. It may be that the enhanced sensitivity relates only to extreme steroid elevation and that adaptation to the sensitivity change does not occur in the time period studied.

A further point of interest stems from the observation that no change was evident in the sensitivity of the vas deferens to catecholamines in animals subjected to irregular signalled escape stress. Failure to observe a change in the sensitivity of this adrenergic system, even though the plasma levels of glucocorticoids were extremely high, may be related to differences in the type of adrenoreceptor being examined. Contraction of the vas deferens in response to adrenergic stimulation is mediated via α-adrenoreceptors [7] whereas the adrenergic response in the myocardium is mediated via β-adrenoreceptors [35]. It is of interest that many of the adrenergic responses involving the β-adrenoreceptor are potentiated by a glucocorticoid-catecholamine interaction. The adrenergic release of glucose from the liver [16,39], increased glycogenolysis in skeletal muscle [38], and increased lipolysis in adipose tissue [23], are examples of responses mediated through this system. Such responses are enhanced in the presence of glucocorticoids [45]. Pun *et al.* [43], in a study of the bronchodilator effects of catecholamines, found that the potent β-receptor agonist isoprenaline was potentiated by hydrocortisone. On the other hand the tension response of the cat nictitating membrane following stimulation of the cervical sympathetic nerve is mediated via α-receptors [7], and is not affected by changes in steroid levels [14]. An effect similar to that described in this paper for the vas deferens.

It is not possible however to postulate that the glucocorticoids only potentiate those actions of the catecholamines mediated through the β-adrenoreceptor. Catecholamine induced constriction of peripheral blood vessels is generally mediated by the α-receptor [35], and potentiation of the pressor effects of catecholamines in the presence of glucocorticoids has been described for certain vascular beds [24, 25, 45, 51].

While the exact nature of the stress-induced change in myocardial sensitivity reported in this paper is not understood, a number of possible mechanisms can be examined. A potentiation of the response mediated by the catecholamines could relate to either a delay in catecholamine inactivation following release from the sympathetic-adrenal medullary system, or enhancement of the sensitivity of the receptor site itself. One method of delaying catecholamine inactivation would be to reduce the efficiency of the enzyme systems associated with the degradation of these amines. Catechol-O-methyl transferase (COMT) is the main extraneuronal enzyme associated with the catabolism of circulating catecholamines [42,47]. In the rat COMT and the intracellular norepinephrine metabolizing enzyme, monoamine oxidase (MAO), play an important role in controlling the accumulation of norepinephrine in cardiac muscle cells [36]. Corticosteroids are reported to inhibit the enzyme COMT [26, 27, 32]. Kalsner and Nickerson [28] reported that COMT was the major pathway for norepinephrine inactivation in the rabbit aorta, and Kalsner [26,27] proposed that it was through an inhibition of COMT that the steroid hormones potentiate the actions of the catecholamines in the rabbit aortic strip. The glucocorticoids however, appear not to affect the activity of COMT in the adrenal gland [50].

Probably the most important mechanism for the inactivation of the catecholamines is their active uptake back into neuronal and extra-neuronal stores [2,30].

Iversen [20] demonstrated the existence of two separate mechanisms for the uptake of norepinephrine into rat heart, $Uptake_1$ and $Uptake_2$. The former mechanism, $Uptake_1$, relates to uptake into adrenergic nerve terminals [20], whereas $Uptake_2$ has been demonstrated to be extra-neuronal [9, 10, 11]. Inhibition by the corticosteroids of the uptake or storage of catecholamines into either neuronal or extra-neuronal stores would increase the availability of the amine to the receptor site. Such an action would result in a potentiation of the catecholamine action.

Iversen and Salt [21] have demonstrated in rat heart a dose dependent inhibition of the $Uptake_2$ mechanism for norepinephrine by various steroids including corticosterone. Nicol and Rae [40] have reported a similar inhibition of the extraneuronal accumulation of epinephrine and norepinephrine produced by the steroid hormones, in arterial smooth muscle. Altered cardiac retention of exogenous norepinephrine, produced by both stress and hydrocortisone, has been reported in young rabbits [14], and Hughes [19], investigating the inactivation of the adrenergic transmitter in the rabbit portal vein and vas deferens following electrical stimulation, concluded that corticosterone blocks the extraneuronal uptake and subsequent metabolism of norepinephrine in these tissues. Cortisone and hydrocortisone inhibit the removal of norepinephrine from the pulmonary vascular space [22]. As a result of these studies, it has been proposed that inhibition of norepinephrine uptake in the lung may potentiate cardiovascular responses to this amine by reducing its pulmonary removal, thus allowing increased concentrations to reach the peripheral arterial circulation.

The other possibility mentioned as a cause of enhanced myocardial sensitivity to catecholamines related to a possible steroid action at the receptor level. This was suggested originally by Besse and Bass [5], following observations that hydrocortisone enhanced the response of the aortic strip to catecholamines. A similar explanation was proposed by Yard and Kadowitz [51] to explain enhanced pressor responses to epinephrine following hydrocortisone. If the basis of the glucocorticoid-catecholamine interaction is at the level of the adrenoreceptor, and as the adrenergic receptors in the heart are of the β type [31,35], it becomes possible to examine the nature of the interaction. Activation of the β-adrenoreceptor is reported to involve the formation of cyclic 3-5 adenosine monophosphate (3-5 AMP) in the effector cell, a substance essential for the conversion of phosphorylase to its active form [46]. It is postulated that formation of cyclic 3-5 AMP by β-receptor activation is a common step both in muscle glycolysis and on the mechanical response of the effector cell. The glucocorticoids are known to increase cardiac but not skeletal muscle phosphorylase activity [17], and to potentiate the adrenaline induced rise in cardiac phosphorylase [15, 17, 18]. It is possible that the glucocorticoids may enhance cardiac phosphorylase activity by facilitating either cyclic 3-5 AMP formation or its activity once formed, and in this way bring about the enhanced myocardial sensitivity to the catecholamines. In this regard chronic stress is known to enhance mitochondrial oxidative and phosphorylative capacities in the heart [48].

In conclusion it can be stated that the results described in this paper demonstrate the existence of enhanced myocardial sensitivity following unpredictable, signalled escape stress, and that this sensitivity is associated with extreme glucocorticoid elevation. However the results discussed do not allow any positive conclusion to be drawn regarding the nature of the enhanced sensitivity, and perusal of the literature indicates there are many experimental parameters which could be directly or indirectly involved.

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