Pharmacology Biochemistry & Behavior, Vol. 4, pp. 39–44.

Effect of Stress on the Uptake of ^{3}H-Norepinephrine into Rat Myocardium[1]

J. R. BASSETT AND K. D. CAIRNCROSS

School of Biological Sciences, Macquarie University, North Ryde, N.S.W., 2113, Australia

(Received 31 March 1975)

BASSETT, J. R. AND K. D. CAIRNCROSS. *Effect of stress on the uptake of ^{3}H-norepinephrine into rat myocardium.* PHARMAC. BIOCHEM. BEHAV. 4(1) 39–44, 1976. – Spontaneously beating atria from rats previously exposed to irregular, signalled footshock were incubated with ^{3}H-norepinephrine. A significant reduction in the uptake and retention of radioactivity was found in the atria from stressed animals compared with unstressed controls. A kinetic study of the uptake process in both the stressed and control groups showed a similar K_m value but a significantly different V_{max}. It was concluded that the enhanced myocardial sensitivity to catecholamines previously reported can be explained in part, on the basis of an inhibition of neuronal uptake.

Stress Catecholamine uptake Uptake inhibition Corticosteroids

BASSETT and Cairncross [1] reported that exposure of male rats to irregular signalled footshock from which the animal could escape resulted in an enhanced myocardial sensitivity to both norepinephrine and epinephrine. On the basis of changes in the endogenous levels of norepinephrine and epinephrine following exposure to stress, an inhibition of the uptake of catecholamines into storage sites was proposed as a possible contributing factor to the enhanced myocardial sensitivity [2]. Furthermore it was suggested that such a stress procedure may inhibit neuronal uptake ($Uptake_1$) as well as extraneuronal uptake ($Uptake_2$). To confirm the presence of a stress induced inhibition of uptake the effect of irregular-signalled escape stress on the myocardial accumulation of ^{3}H-norepinephrine by spontaneously beating isolated rat atria, was measured following various periods of incubation.

In order to clarify which uptake mechanism was affected by stress a kinetic study was undertaken. Kinetic analysis of both the $Uptake_1$ and $Uptake_2$ mechanism show both qualitative and quantitative difference [4, 8, 9, 10]. These differences in kinetic properties act to characterise each uptake system. $Uptake_1$ is operative at low catecholamine concentrations whereas $Uptake_2$ becomes more apparent at high concentrations. Both processes saturate with increasing external concentration and can be described by Michaelis-Menten kinetics. The affinity of (±)-norepinephrine for $Uptake_2$ is 374 times less than its affinity for $Uptake_1$, however with $Uptake_2$, norepinephrine uptake is rapid and is completed in a short time [10]. In this study the initial rates of norepinephrine uptake were measured at various incubation concentrations of norepinephrine. The results were subjected to a Michaelis-Menten analysis from which the kinetic constants K_m and V_{max} were determined. The kinetic constants were used to identify the uptake process most affected by the stress procedure.

The contribution of ^{3}H-norepinephrine to the total radioactivity obtained from homogenated rat atria was determined using chromatographic separation. Iversen [11] suggested that circulating transmitter removed by $Uptake_2$ may subsequently suffer extraneuronal metabolism. Any changes in the proportions of unchanged norepinephrine or its metabolites may indicate inhibition of metabolic enzymes which, together with the inhibition of uptake, may play a role in the stress induced enhanced myocardial sensitivity.

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. – 8p.m.) beginning at least 14 days prior to commencement of experimentation. Food and water were provided ad lib. Both control and stressed rats were housed under identical conditions.

Stress Apparatus and Procedure

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 2 W was located on the wall of the grid chamber opposite to the escape platform. The

[1]The skilled technical assistance of Mrs. Carol Martin is gratefully acknowledged. The work reported in this paper was supported by Grant number D1 73/15017 from the Australian Research Grants Committee.

unconditioned stimulus (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. Treatment consisted of 7 CS-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. Animals were stressed daily, one session/day, for 4 days. Immediately after completion of the last stress period, 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. Plasma corticosterone levels were determined subsequently by the fluorimetric method of Mattingley [14], which is specific for free 11-hydroxycorticosteroids.

Tissue Preparation

The atria were suspended in a 15 ml organ bath containing Krebs-Henseleit solution gassed with 5 percent carbon dioxide in oxygen and maintained at 29°C. Atropine sulphate at a concentration of 2.9×10^{-8} M was added to the Krebs solution. The inotropic response of the spontaneously beating atria was monitored using an isotonic strain gauge transducer and recorded on a Cardiotrace recorder. Preparations were allowed to equilibrate for 30 min after setting up before commencement of the experiment. Ethylenediamine tetra acetic acid (EDTA), 20 μg/ml, was added to the solution in the organ bath 5 min before the addition of norepinephrine. Cumulative log dose-response curves for norepinephrine were obtained and the ED_{50} was derived from the 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 for linearity and the ED_{50} calculated using regression coefficients. The ED_{50}'s from 7 experiments were measured and the mean ED_{50} calculated. This concentration of norepinephrine was used in the study of accumulation of radioactivity in the myocardium as a function of the time of incubation.

Accumulation of radioactivity as a function of time. Control and stressed atria were incubated with ^{3}H-7-1-norepinephrine (1×10^{-8} g/ml in organ bath; 5.92×10^{-8} M) for periods of 2, 5, 7, 10, 15 and 20 min. The ^{3}H-norepinephrine (Specific activity 8.7 Ci/mmole) was obtained chromatographically pure from the Radiochemical Centre, Amersham (England). Each batch of ^{3}H-norepinephrine was standardized. Following incubation with the labelled amine the atria were removed from the organ bath, washed in Krebs-Henseleit solution and blotted, this procedure being repeated 3 times. The tissues were then weighed, and homogenized in 3 ml of 0.4 N perchloric acid containing 10 μg/ml cold norepinephrine. The homogenate was centrifuged at 5,000 rpm for 15 min, then 200 μl of the supernatant was added to 15 ml of scintillation solution in a counting vial. The scintillation solution was prepared by mixing 350 ml of ethanol with 650 ml of toluene solution containing 4.0 g/l of 2,5-diphenyloxazole (PPO) and 0.1 g/l of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP). All samples were counted for 10 min periods in a Packard TRI-CARB liquid scintillation counter. Duplicate samples were taken from each homogenate and the counts obtained from the duplicates were meaned. Background blanks were prepared for each experimental series by setting up atria and performing all procedures detailed above with the exception that no ^{3}H-norepinephrine was added to the incubation fluid. The automatic external standarization ratio technique was used to monitor quenching. Efficiency curves were obtained and all counts were corrected for counting efficiency to give the number of disintegrations/min (DPM). The DPM's due to background activity (Blank) were subtracted from the mean DPM obtained from the duplicates for each homogenate. Total tissue radioactivity was then calculated at DPM/mg of atrial tissue after correcting for the water content of the atria. The accumulation of radioactivity in atria from both stressed and control animals after various incubation periods was compared using unpaired *t* tests.

Initial rate of norepinephrine uptake at various incubation concentrations. The method was essentially the same as that described above, except that in this series of experiments the incubation time was fixed at 5 min and the concentration of ^{3}H-7-norepinephrine (specific activity 7.8 Ci/mmole) was varied. At high concentrations cold l-norepinephrine was added to the bath to make up the required concentration. The DPM measured was corrected accordingly. Results were expressed as DPM/mg/min.

In order to see if the norepinephrine uptake obeyed Michaelis-Menten kinetics, the initial rate of norepinephrine uptake at the various concentrations of norepinephrine was plotted in the form S/v against S (S = incubation concentration of norepinephrine (molar bath concentration), v = initial rate of norepinephrine uptake (DPM/mg/min). From the slopes and intercepts of these graphs the kinetic constants V_{max} and K_m were determined [8].

Chromatographic separation of total radioactivity into its component parts. Atria from control and stressed animals were incubated with 7.96×10^{-8} M (1.35×10^{-8} g/ml) ^{3}H-7-1-norepinephrine for 5 min. The atria were then washed, blotted and weighed as described above, then homogenized in 3 ml of 75 percent ethanol containing 0.1 mg of each of the following non-radioactive substances; l-norepinephrine (NA), normetanephrine (NM), 3-methoxy-4-hydroxymandelic acid (MHMA), 3,4-dihydroxymandelic acid (DHMA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3,4 dihydroxyphenylglycol (DHPG). These substances have been shown to be the major metabolites of norepinephrine in rat atria [16]. The homogenate was stored overnight at −20°C then centrifuged at 5,000 rpm for 15 min. An aliquot of the supernatant (100 μl) was taken and spotted as a 3 cm line onto aluminium thin layer chromatography plates precoated with cellulose (Merck; layer thickness 0.1 mm). Spots were dried in cold air. The chromatograms were run for 4 hr in a solvent mixture n-butanol: 5 N acetic acid (100:35). The solvent system gave a good separation of all the components in question. The chromatograms were oven dried at 80°C for 15 min then sprayed with a mixture of 0.1 percent p-nitroaniline, 0.2 percent sodium nitrite, and 10 percent potassium carbonate in the ratio 1:1:2 in order to visualize the position of the standards. The TLC plates containing the homogenate and cold carriers were cut into segments containing the visualized spots and the region between the

TABLE 1

THE ACCUMULATION OF RADIOACTIVITY IN ISOLATED SPONTANEOUSLY BEATING RAT ATRIA FOLLOWING INCUBATION WITH ^{3}H-7-L-NOREPINEPHRINE

Incubation Time (min)	Uptake of Radioactivity (DPM/mg) mean ± SE Control	Stress	*t* Test *p*
2	488 ± 23 (10)	394 ± 27 (9)	<0.001
5	1112 ± 35 (12)	879 ± 34 (12)	<0.001
7	1430 ± 85 (6)	1023 ± 69 (6)	<0.001
10	1899 ± 51 (6)	1766 ± 92 (6)	>0.05
15	2665 ± 202 (6)	2164 ± 102 (6)	<0.01
20	3584 ± 268 (6)	3328 ± 97 (6)	>0.40

$p<0.05$ is significant ( ) = number of animals/group

origin and the first visualized spot. The cellulose was scraped from each segment into counting vials. The radioactive isotopes were extracted in the sealed vials with 4 ml of methanol overnight, then counted after the further addition of 10 ml of scintillation mixture (4g/l POP, 0.1 g/l POPOP in toluene). With the exception of the segments containing the origin and visualized spots all other areas of the plate were found to contain negligible activity above background.

The counts were corrected for background and for efficiency of counting. Results were expressed as the percent of radioactivity in each of the individual segments (in DPM) using total activity on the TLC plate as 100 percent.

RESULTS

The mean ED_{50} (± S.E.) for l-norepinephrine on spontaneously beating rat atria, determined using 7 animals, was $5.74 (\pm 0.77) \times 10^{-8}$ M. Spontaneously beating atria were incubated with ^{3}H-7-l-norepinephrine at a dose approximating the ED_{50} (5.92×10^{-8} M) for various time periods. This dose was chosen since the changes in myocardial sensitivity observed by Bassett and Cairncross [1] were measured as changes in the ED_{50} for norepinephrine and epinephrine. Therefore mechanisms that influence myocardial sensitivity at this dose level of norepinephrine were the ones of immediate interest.

The accumulation of radioactivity in atria from stressed and unstressed animals is shown in Table 1. A significant reduction in the accumulation of radioactivity was observed in the atria from stressed animals following incubation for 2, 5, 7 and 15 min. No significant reduction was observed following incubation for 10 and 20 min. The mean plasma 11-hydroxycorticosteroid level (± S.E.) in control animals was 20.8 ± 1.4 μg/100 ml plasma compared with the mean level in stressed animals of 90.3 μg/100 ml plasma. The value of plasma steroid elevation in the stressed animals agrees well with the levels reported by Bassett *et al.* [3] and Bassett and Cairncross [1] following 4 days exposure to the same stressor (89 ± 4.9 and 91 ± 4.8 μg/100 ml plasma respectively).

The initial rate of accumulation of radioactivity at various concentrations of ^{3}H-norepinephrine is shown in Table 2. At low incubation concentrations (1.59, 3.18 and 4.78×10^{-8} M) there was no significant difference between the atria from stressed and control animals in their initial rate of uptake of radioactivity, although the values for stressed atria tended to be lower. At higher incubation concentrations (6.35×10^{-8} to 39.78×10^{-8} M) the initial rate of uptake was significantly less in atria from stressed animals. These results were plotted in a form of the Michaelis-Menten equation as S/v against S (Fig. 1). While the values for stressed atria tended to lie on or close to a straight line, as would be expected if Michaelis-Menten kinetics were obeyed, the control values at low incubation concentrations tended to deviate sharply towards the stress kinetic curve. These points corresponded to the incubation concentrations where no significant difference occurred in the initial rate of uptake between atria from control and stressed animals. As a result of the marked difference between the initial values and the rest of the control values, the points corresponding to low incubation concentrations of norepinephrine were not included in Fig. 1 nor in the kinetic analysis. The significance of this failure to obey Michaelis-Menten kinetics in the controls at low concentrations of norepinephrine will be discussed later. Linear regression analysis of the results from stressed and control atria gave straight lines with the following correlation coefficients, r = 0.996 (control) and r = 0.987 (stressed). The values of K_m calculated using the regression equations (±95 percent confidence limits) were 59.2 (±13.7) $\times 10^{-8}$ M for control atria, and 58.7 (± 6.1) $\times 10^{-8}$ M for stressed atria. A test for parallelism on the two regression equations indicated that the two lines were not parallel, the

TABLE 2

INITIAL RATE OF UPTAKE OF RADIOACTIVITY IN ISOLATED SPONTANEOUSLY BEATING RAT ATRIA FOLLOWING INCUBATION WITH ^{3}H-7-L-NOREPINEPHRINE

Concentration of Norepinephrine ($\times 10^{-8}$ M)	Initial Rate of Uptake (DPM/mg/min) mean ± SE		*t* Test *p*
	Control	Stressed	
1.59	109 ± 11 (6)	108 ± 7 (5)	>0.80
3.18	223 ± 20 (5)	202 ± 14 (5)	>0.40
4.78	345 ± 23 (5)	273 ± 19 (5)	>0.05
6.35	517 ± 15 (5)	393 ± 43 (5)	<0.02
7.96	630 ± 29 (5)	455 ± 23 (5)	<0.005
15.91	1158 ± 40 (5)	840 ± 67 (5)	<0.005
23.87	1500 ± 53 (5)	1112 ± 79 (5)	<0.01
39.78	2187 ± 67 (5)	1620 ± 159 (5)	<0.01

( ) = number of animals/group

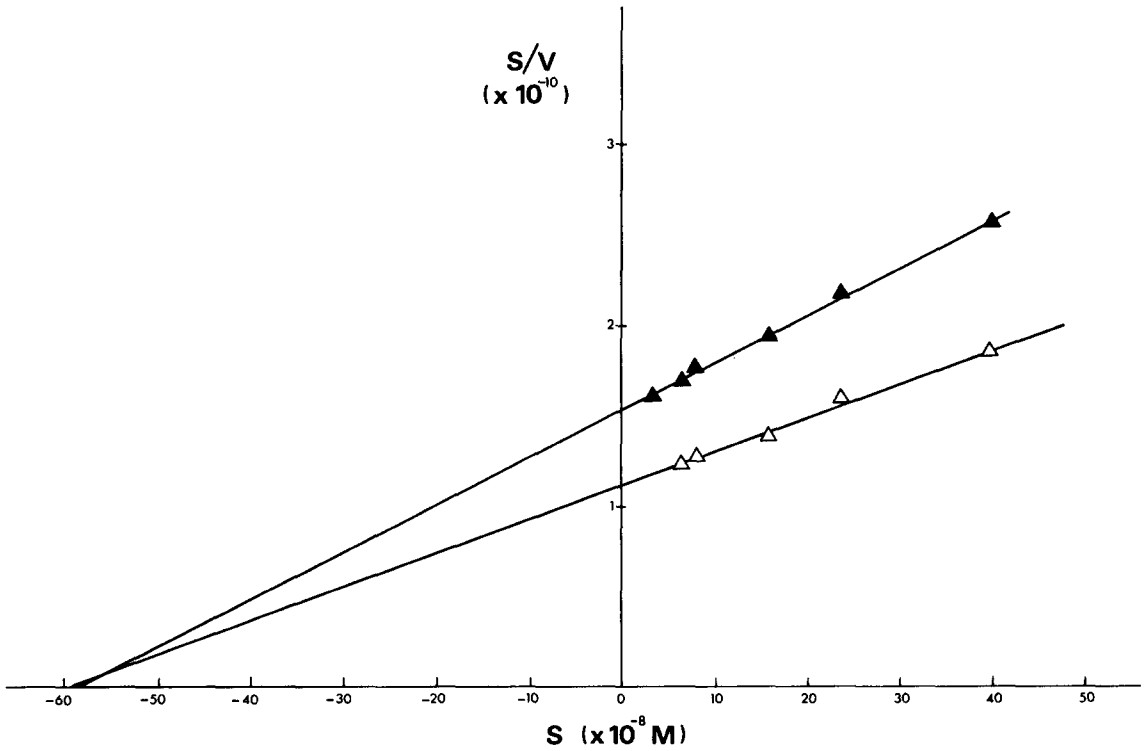

FIG. 1. Michaelis-Menten analysis of uptake results. S = incubation concentration of norepinephrine (molar bath conc.). v = initial rate of norepinephrine uptake by atria (DPM/ml/min). Intercept on baseline = $-$Km. Slope = $1/V_{max}$. ▲ = Stressed animals. △ = Control animals.

TABLE 3

THE PROPORTION OF TOTAL RADIOACTIVITY CONTRIBUTED BY NOREPINEPHRINE AND ITS METABOLITES

Compound	R_f Value (± SE)	Percent of Total Activity (± SE) Control	Stressed	*t* Test *p*
Conjugated forms	–	5.7 ± 1.7	6.2 ± 0.9	>0.70
Norepinephrine	0.212 ± 0.006	79.9 ± 2.0	77.7 ± 1.3	>0.30
Normetanephrine	0.330 ± 0.004	4.5 ± 0.3	3.2 ± 0.4	<0.02
Dihydroxy metabolites	0.529 ± 0.003	5.3 ± 0.5	7.6 ± 0.7	<0.05
Methoxyhydroxy metabolites	0.731 ± 0.006	4.7 ± 0.4	5.3 ± 0.3	>0.20

two gradients (V_{max} stressed and control) being significantly different ($p<0.001$).

V_{max} (control) = 5322 DPM/mg/min

V_{max} (stressed) = 3785 DPM/mg/min

In the chromatographic separation of norepinephrine and its metabolites from both control and stressed animals 4 separate bands were visualized. The R_f values of these bands agreed well with the values obtained for the standards NE, NM, MHPG, DHPG, MHMA, and DHMA and indicated that the bands in ascending order of R_f contain norepinephrine, normetanephrine, the dihydroxy metabolites DHMA and DHPG, and finally the methoxyhydroxy metabolites MHMA and MHPG. Activity remaining close to the origin probably resides in the conjugated forms of norepinephrine and its metabolites. The proportion of the total activity found in the visualized bands and the origin are shown in Table 3. It can be seen that in both the control and stressed atria unchanged norepinephrine contributes by far the greatest proportion of the total activity. The proportions of conjugates, norepinephrine and methoxyhydroxy metabolites were not significantly affected by the stress procedure. The proportion of normetanephrine however, was significantly lowered in stressed animals, while the dihydroxy metabolites were significantly elevated.

DISCUSSION

The inhibition of uptake of norepinephrine following exposure to irregular-signalled escape stress, suggested by Bassett and Cairncross [2], is confirmed by this study. The accumulation of ^{3}H-norepinephrine was significantly reduced in atria from stressed animals. The inhibition, however, appears to reflect an interference in the rate of uptake into storage sites rather than a change in the storage itself. While the accumulation of radioactivity was initially less in stressed atria, if the incubation time was prolonged the levels of activity in tissue from both control and stressed animals were not significantly different. This finding is confirmed by the kinetic studies. Atria from stressed animals showed a significant lowering of the maximum rate of uptake (V_{max}) but no change in the affinity of norepinephrine for the uptake mechanism. These results may be interpreted on the basis that in both control and stressed preparations the norepinephrine is competing for the same uptake sites but that in the stress situations there may be fewer sites available. The reduction in available uptake sites may reflect the binding to such sites of 11-hydroxycorticosteroids, ACTH or some other unknown compound liberated in response to stress or to a change in receptor molecule turnover.

The fact that the concentrations of norepinephrine used in this study were well within the range of concentration found by Iversen [8, 10, 11] and Farnedo and Malmfors [5] to be accumulated almost exclusively by Uptake1, suggests that it is mainly neuronal uptake which is affected by exposure to stress. Again such an hypothesis is confirmed by the kinetic studies. The K_m for control and stressed tissues were 59.2×10^{-8} M and 58.7×10^{-8} M respectively. Iversen [8] found that for $Uptake_1$ the K_m for (−) norepinephrine was 26.6×10^{-8} M but that a significant stereochemical specificity existed, (+)-norepinephrine giving a K_m of 139.0×10^{-8} M and (±)-norepinephrine 66.4×10^{-8} M. The affinity of norepinephrine for $Uptake_2$ is considerably less, the K_m for (±)-norepinephrine being 25.2×10^{-5} M with no significant stereochemical specificity being demonstrated [10]. These findings do not mean that the $Uptake_2$ mechanism is not affected in stress situations but simply that an inhibition of $Uptake_1$ (neuronal uptake) does appear to occur and that such an inhibition would explain the changes in myocardial sensitivity described by Bassett and Cairncross [1]. On the basis of the reports by Iversen and Salt [12], Nicol and Rae [15] and Hughes [6], inhibition of $Uptake_2$ may well occur but will only be apparent when the level of catecholamine and corticosteroid at the uptake site is high.

The failure of the accumulation of ^{3}H-norepinephrine to obey Michaelis-Menten kinetics at low concentrations is of interest. The deviation from the expected values really only applies to the control atria and deviation decreases with increasing concentrations. Hughes [7] demonstrated a differential labelling of neuronal norepinephrine stores with different concentrations of ^{3}H-l-norepinephrine. Hughes suggested that exogenous ^{3}H-norepinephrine may not mix homogenously within neuronal stores but that at

low concentrations, exogenous norepinephrine is taken up into stores from which it can be easily released. At higher concentrations of ^{3}H-norepinephrine homogenous labelling of tissue stores can be achieved and the norepinephrine more firmly bound. Loss of ^{3}H-norepinephrine from such easily releasable neuronal stores would explain why a reduced accumulation of radioactivity was observed at low incubation concentrations in the controls. At higher concentrations the ^{3}H-norepinephrine is taken up into stores from which it is not so readily removed and, therefore, at these concentrations the accumulation of radioactivity more accurately reflects the uptake process. Since neuronal uptake is inhibited in stressed animals such a differential storage may not be so apparent.

An inhibition of neuronal uptake would be expected to enhance the availability of norepinephrine to the extraneuronal metabolizing enzyme COMT and thus result in an elevation of O-methylated metabolites in stressed atria. The small but significant decrease in the proportion of normetanephrine observed in atria from stressed animals therefore, may be indicative of an inhibition of COMT. Such an inhibition of this extraneuronal enzyme would supplement the already enhanced availability of norepinephrine at the receptor site and thus contribute to the stress induced change in myocardial sensitivity. The significant increase in the level of dihydroxy metabolites following exposure to stress may be explained by an increased activity of the intraneuronal enzyme MAO, secondary to an increased norepinephrine synthesis rate. Westfall and Osada [17] have suggested that an increased sympathetic nerve activity, as occurs in stress situations [13], results not only in an increased synthesis rate of norepinephrine but also an increased MAO activity.

REFERENCES

1. Bassett, J. R. and K. D. Cairncross. Myocardial sensitivity to catecholamines following exposure of rats to irregular, signalled footshock. *Pharmac. Biochem. Behav.* **4:** 27–33, 1976.
2. Bassett, J. R. and K. D. Cairncross. Endogenous levels of catecholamines in the rat myocardium following exposure to stress. *Pharmac. Biochem. Behav.* **4:** 35–38, 1976.
3. Bassett, J. R., K. D. Cairncross and M. G. King. Parameters of novelty, shock predictability and Response Contingency in Corticosterone Release in the Rat. *Physiol. Behav.* **10:** 901–907, 1973.
4. Burgen, A. S. V. and L. L. Iversen. The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart. *Br. J. Pharmac.* **25:** 34–49, 1965.
5. Farnedo, L. and T. Malmfors. Histochemical studies on the uptake of noradrenaline and α-methyl-noradrenaline in the perfused rat heart. *Eur. J. Pharmac.* **5:** 313–320, 1969.
6. Hughes, J. Evaluation of mechanisms controlling the release and inactivation of the adrenergic transmitter in the rabbit portal vein and vas deferens. *Br. J. Pharmac.* **44:** 472–491, 1972.
7. Hughes, J. Differential labelling of intraneuronal noradrenaline stores with different concentrations of (−)-^{3}H-noradrenaline. *Br. J. Pharmac.* **47:** 428–430, 1973.
8. Iversen, L. L. The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac.* **21:** 523–537, 1963.
9. Iversen, L. L. The uptake of adrenaline by the rat isolated heart. *Br. J. Pharmac.* **24:** 387–394, 1965.
10. Iversen, L. L. The uptake of catecholamines at high perfusion concentrations in the rat isolated heart: a novel catecholamine uptake process. *Br. J. Pharmac.* **25:** 18–33, 1965.
11. Iversen, L. L. Role of transmitter uptake mechanisms in neurotransmission. *Br. J. Pharmac.* **41:** 571–591, 1971.
12. Iversen, L. L. and P. J. Salt. Inhibition of catecholamine Uptake_2 by steroids in the isolated rat heart. *Br. J. Pharmac.* **40:** 528–530, 1970.
13. Mason, J. W. A review of psychoendocrine research on the sympathetic adrenal medullary system. *Psychosom. Med.* **30:** 631–653, 1968.
14. Mattingley, D. A simple fluorimetric method for the estimation of free 11-hydroxy-corticosteroids in human plasma. *J. clin. Pathol.* **15:** 374–379, 1962.
15. Nicol, C. J. M., and R. M. Rae. Inhibition of accumulation of adrenaline and noradrenaline in arterial smooth muscle by steroids. *Br. J. Pharmac.* **44:** 361–362, 1972.
16. Roffler Tralov, S. and S. Z. Langer. The fate of ^{3}H-norepinephrine released from isolated atria and vas deferens: effect of field stimulation. *J. Pharmac. exp. Ther.* **179:** 186–197, 1971.
17. Westfall, T. C. and H. Osada. Influence of adrenalectomy on the synthesis of noradrenaline in the rat heart. *J. Pharmac. exp. Ther.* **167:** 300–308, 1969.