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EFFECTS OF HYPOTHERMIA AND ANOXIA ON RETENTION OF NORADRENALINE BY THE CAT PERFUSED HEART

BY

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Considerable evidence has accumulated in recent years to indicate that the cardiovascular response to noradrenaline, either released after sympathetic nerve stimulation or administered by injection, is terminated by tissue uptake and subsequent binding of the amine (Whitby, Axelrod & Weil-Malherbe, 1961; Haefely, Hurlimann & Thoenen, 1964). Many drugs that decrease or prevent such functional inactivation of noradrenaline also produce hypersensitivity to the amine; these findings may be causally related (Trendelenburg, 1963, 1965; Haefely *et al.*, 1964).

Anoxia (Gowdey & Patel, 1964) or prolonged infusion of noradrenaline (Rosenthale & Dipalma, 1963) cause a decreased sensitivity to the amine, while hypothermia potentiates the response to sympathetic nerve stimulation of cat isolated atria (Schneider & Gillis, unpublished) and guinea-pig vas deferens preparations (Della Bella, Gandini & Preti, 1965). In these instances of altered cardiovascular reactivity to noradrenaline, it seemed possible that the mechanism might involve an alteration in the ability of tissues to bind and thus inactivate the amine. Accordingly we have studied the effect of anoxia and hypothermia on the ability of heart to retain [^{3}H]-noradrenaline. For this purpose, the cat isolated heart, perfused at a constant flow rate, has been used. Paton & Gillis (1965) have shown that the retention of [^{3}H]-noradrenaline by cat spleen is inversely related to the perfusion rate; this observation has now been extended to include the perfused heart.

METHODS

Perfusion technique

Cats of either sex weighing between 1.8 and 4.2 kg were anaesthetized with pentobarbitone sodium (30 mg/kg). The trachea was cannulated and respiration was maintained by means of a Palmer pump. The heart was prepared for perfusion, using a modification of the Langendorff method. The heart and great vessels were exposed by means of a right thoracotomy. The arch of the aorta was incised as far as possible from the aortic valves and the vessel was then rapidly cannulated with a curved glass cannula. The cannula in turn was connected by tubing to a constant outflow perfusion pump (Sigmamotor Model No. TM 11). The flow of perfusion fluid (Krebs solution at 37° C containing 10 μg/ml. of sodium edetate and equilibrated with 95% oxygen and 5% carbon dioxide, unless otherwise indicated) was started before cannulation. After cannulation, the heart was rapidly removed from the thorax and suspended in air surrounded by a glass jacket through which flowed water at 37° C. Unless otherwise stated, the perfusion rate was 14 ml./min. A fine

cotton thread connected the apex of the left ventricle to a force-displacement transducer (Statham Instrument Co.), the output of which was recorded on a Gilson polygraph. The heart rate was measured directly from the record. Those hearts which failed to beat regularly within 15 min of cannulation were discarded.

During each test period, a total of 140 ng of [^{3}H]-noradrenaline and 2.66 μg of non-isotopic noradrenaline was infused into the rubber tubing proximal to the perfusion pump, by means of an infusion pump (Harvard Instrument Co., Model 600–960). The duration of each infusion was either 1 or 20 min and the rate of delivery of amine to the perfusion fluid was such that the final concentration of noradrenaline in the medium was 200 or 10 ng/ml. respectively. Each heart received an infusion of noradrenaline under control conditions and an identical infusion under the experimental condition being examined. The order varied from heart to heart, but in all experiments at least 30 min elapsed between the two successive infusions of noradrenaline.

Production of hypothermia

The temperature of the heart was lowered by perfusing Krebs solution at 29° C and also by cooling the water circulating through the glass jacket surrounding the heart to the same temperature. Hearts were allowed to adjust to the reduced temperature for at least 30 min before the infusion of noradrenaline.

Production of anoxia

In these experiments, perfusion was carried out with Krebs solution previously equilibrated with 95% nitrogen and 5% carbon dioxide and kept at 37° C. After 6 min of anoxia had elapsed the infusion of noradrenaline was started.

Measurement of [^{3}H]-noradrenaline retention

The effluent fluid from the heart was collected for 1-min periods during each of the 3 min before, during and for each of the 20-min periods immediately after stopping the infusion. The volume of each sample was noted and the tritium of a 0.1-ml. aliquot was measured, after mixing with 10 ml. of Bray's phosphor (Bray, 1960), using a Packard Tri-Carb liquid scintillation counter. The over-all counting efficiency for tritium in this instrument was 25%. Quenching, determined by means of tritiated water internal standards, ranged from 20 to 25% and was neglected in the calculation of total tritium in each sample of effluent. Retention of [^{3}H]-noradrenaline was taken as the difference between the total amount of [^{3}H]-noradrenaline infused (assessed by direct measurement of tritium) and the total amount of tritium recovered in the venous effluent during and for 15 min after the infusion. At least 80% of the tritium recovered was found in the venous effluent within 6 min of terminating the infusion. In some experiments, the [^{3}H]-noradrenaline content of the effluent, during and immediately after an infusion of amine, was determined by the method of Schneider & Gillis (1965), and was found to be over 80% of the total tritium measured.

Drugs

Chromatographically pure (±)-noradrenaline-7-[^{3}H]-hydrochloride (in 0.1 N-acetic acid) with a specific activity of 4.72 c/mmole was obtained from the New England Nuclear Corporation. The solution of [^{3}H]-noradrenaline was diluted with 0.2 N-hydrochloric acid (final concentration 0.001 N) and 10% sodium metabisulphite (final concentration 1%) and glass distilled water to give a stock solution containing 10 μg/ml. of the base, which was stored at 4° C. (±)-Noradrenaline hydrochloride (Calbiochem) was prepared as a stock solution containing 1.0 mg of the base per ml. of 0.2 N-hydrochloric acid and was also stored at 4° C. Other drugs used were: atropine sulphate, bretylium tosylate (Burroughs Wellcome) and reserpine (Ciba).

Statistical analysis

When the significance of data was evaluated, Student's *t*-test for paired data was the method used (Bernstein & Weatherall, 1952). Where groups of unequal size were compared, the weighted *t*-test was used (Johnson, 1949).

RESULTS

Effects on [³H]-noradrenaline retention

Hypothermia. In all experiments, reduction of the temperature of the perfusion medium and of the heart itself to 29° C produced a considerable bradycardia ; in many instances spontaneous arrythmias were also seen. Accompanying the hypothermia was a consistent reduction in the retention of [³H]-noradrenaline (Table 1), that was highly significant ($P<0.001$) at a flow rate of 14 ml./min. At a flow rate of 7 ml./min retention of [³H]-noradrenaline was also reduced in each of the three experiments in which this flow rate was used. It should be emphasized that there was still considerable retention of the amine at the lower temperature. In many experiments hypothermia caused an apparent potentiation (see Discussion) of the increase in rate after the amine infusion.

TABLE 1

EFFECT OF TEMPERATURE AND PERFUSION RATE ON THE RETENTION OF [³H]-NORADRENALINE BY THE ISOLATED PERFUSED HEART

Noradrenaline, 2.66 mg, and [³H]-noradrenaline, 140 ng, were infused at a rate chosen to achieve a total amine concentration of 200 ng/ml.

Perfusion rate (ml./min)	Duration of infusion (min)		Retention (%) of [³H]-noradrenaline			*P*	
			At 37° C	At 29° C	Difference	Paired *t*-test	Weighted *t*-test
7	2		74·2	65·6	−8·6		
			64·3	62·1	−2·2		
			64·2	53·9	−10·3		
		Mean and standard error	67·6±3·3 (A)	60·5±3·5 (B)	−7·0±2·5	A *v.* B <0·20	A *v.* C <0·02
14	1		63·0	59·4	−3·6		
			20·4	17·8	−2·6		
			40·1	37·8	−2·3		
			51·4	43·6	−7·8		
			28·3	25·2	−3·1		
			44·6	38·8	−5·8		
			24·2	17·7	−6·5		
		Mean and standard error	38·9±5·9 (C)	34·3±5·7 (D)	−4·5±0·8	C *v.* D <0·001	B *v.* D <0·05

Anoxia. All the anoxic hearts exhibited bradycardia before the infusion of noradrenaline. With persistence of the anoxic state, many of the hearts became very arrythmic and, particularly during the noradrenaline infusion, some stopped beating. In view of these pronounced abnormalities, it was impossible to conclude that anoxia had any definite effect on the chronotropic response of the heart to noradrenaline. It is apparent from Table 2, however, that anoxia had no significant effect on the retention of [³H]-noradrenaline. During periods of anoxia, as in the control periods, [³H]-noradrenaline made up over 80% of the total tritium in the effluent, during and immediately after the infusion of the amine.

Tolerance to the noradrenaline infusion. In a total of four experiments, the initial tachycardia following the start of a prolonged (20 min) noradrenaline infusion was not

TABLE 2

LACK OF EFFECT OF ANOXIA ON THE RETENTION OF [^{3}H]-NORADRENALINE BY THE ISOLATED PERFUSED HEART

Each value is the mean and standard error of five determinations. Noradrenaline, 2·66 mg, and [^{3}H]-noradrenaline, 140 ng, were infused during each experimental period at a constant perfusion rate of 14 ml./min at 37° C. *P*-values are by the paired *t*-test

Duration of infusion (min)	Concentration of amine infused (ng/ml.)	Retention (%) of [^{3}H]-noradrenaline		
		Oxygenation	Anoxia	*P*
1	200	34·0±5·2	36·1±4·3	<0·50
20	10	48·2±6·3	51·3±6·7	<0·20

sustained despite the continuing presence of the amine in the perfusion fluid. These experiments, of which the results illustrated in Fig. 1 are representative, afforded an opportunity of examining the relationship between retention of [^{3}H]-noradrenaline and tolerance to the amine. It can be seen in Fig. 1 that, at a time when the initially elevated heart rate was falling, the amount of tritium appearing in the venous effluent was unchanged, implying that retention was unrelated to the reduced physiological response to noradrenaline.

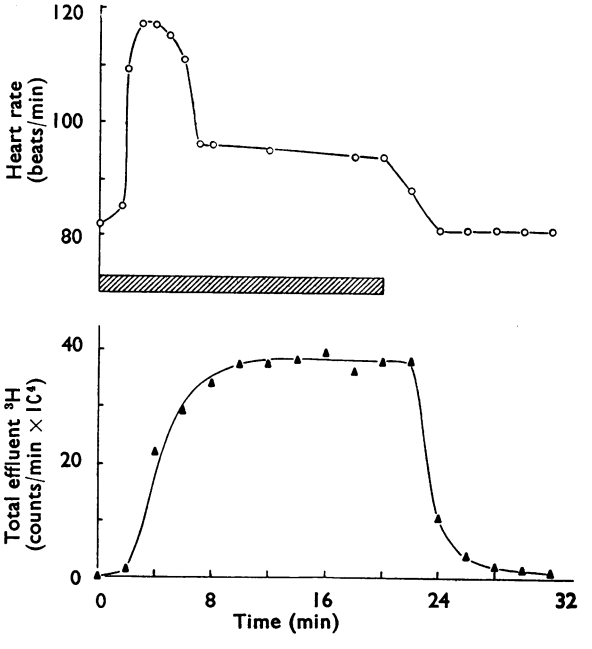

Fig. 1. The influence of tolerance to the chronotropic effect of a sustained noradrenaline infusion (1.8 mg) on the retention of [^{3}H]-noradrenaline (1.0 mg). Infusion was during the period of 20 min indicated by the hatched rectangle. The total tritium (of which 80% was associated with noradrenaline) is a measure of amine retention by the heart. The results illustrated are representative of those obtained in four similar experiments.

Effects of altered perfusion rate

Heart rate. As shown in Table 1, the effect of hypothermia on [^{3}H]-noradrenaline retention was studied at two different flow rates. During these experiments, it became

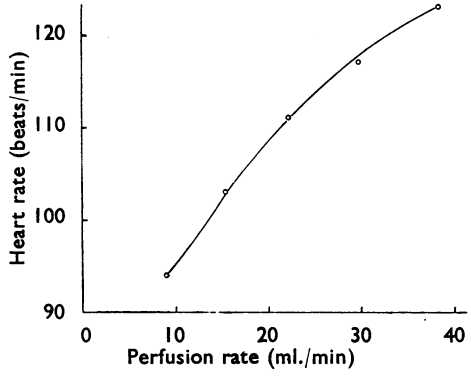

Fig. 2. The relationship between perfusion and heart rates. This figure is representative of ten similar experiments.

clear that a decrease in the perfusion rate produced a consistent and rapid decrease in the heart rate, which was reversible upon resumption of the original higher flow rate. This observation had not been reported previously and therefore merited further investigation. Accordingly, the influence of a wide range of flow rates on heart rate was examined at four or five different flow rates in each of ten hearts. Fig. 2 shows that a direct relationship exists between heart rate and the perfusion rates used. The relationship was uninfluenced by previous treatment of the animals, 48 and 24 hr before use, with reserpine (0.5 mg/kg, intraperitoneally), or by addition to the perfusion fluid of atropine sulphate (10 μg/ml.) or bretylium tosylate (20 μg/ml.). There was a strong sympathomimetic response when bretylium was added to the perfusion fluid; however, the heart rate still increased after the rate of perfusion was raised. The relationship also persisted in those experiments in which the heart was cooled to 29° C. It seemed

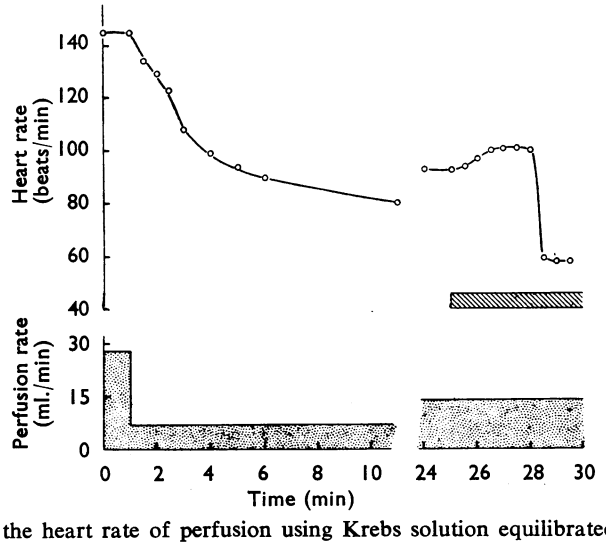

Fig. 3. The effect on the heart rate of perfusion using Krebs solution equilibrated with 95% nitrogen and 5% carbon dioxide (during period indicated by the hatched rectangle). The Krebs solution was equilibrated with 95% oxygen and 5% carbon dioxide before the anoxic equilibration. Results illustrated are representative of five similar experiments.

possible that lower perfusion rates might decrease the heart rate by lowering the rate of delivery of dissolved oxygen or glucose to the myocardium. When Krebs solution equilibrated with 95% nitrogen and 5% carbon dioxide was used as the perfusion fluid, the heart rate was reduced, but not until 3 min after the beginning of the anoxic period (Fig. 3). This observation should be contrasted with the immediate drop in heart rate following a reduction of the rate of perfusion, which is also shown in Fig. 3. In two experiments, the concentration of glucose in the perfusion fluid was altered so that the amount of glucose delivered to the heart per min at each of four different flow rates was maintained constant; despite this, however, the heart rate was still influenced in the same way by alteration of the flow rate.

Retention of [^3H]-noradrenaline. It can be seen in Table 1 that increasing the perfusion rate from 7 to 14 ml./min reduced by almost one-half the retention of [^{3}H]-noradrenaline. The reduction was significant whether the temperature during the experiment was 37° C ($P<0.02$) or 29° C ($P<0.05$).

DISCUSSION

The Langendorff technique that is commonly used involves retrograde perfusion of the aorta with fluid under a constant pressure. Since the technique we have used differs in several fundamental aspects from the original method, it is appropriate to consider the reasons for our use of these modifications. By maintaining the animals on artificial respiration before aortic cannulation, the duration of myocardial anoxia usually associated with death of the animal and the aortic cannulation was considerably reduced, if not completely avoided. Since there was little danger of myocardial anoxia developing, the preparation for cannulation could be carried out more carefully and the position of the cannula, relative to the aortic valves, could be more precisely assessed. The avoidance of myocardial anoxia during preparation was clearly of particular importance in view of our aim to study controlled anoxia as a determinant of [^{3}H]-noradrenaline retention. As already noted in Methods, the flow of perfusion fluid was started before aortic cannulation. This precaution, together with the fact that the heart was pumping blood out of the aortic incision during cannulation, minimized the chance of air embolism in the coronary system.

The use of a constant outflow pump for perfusion of the coronary vasculature reduced to a minimum changes in perfusion fluid flow rate that were due to drugs or other procedures used. The maintenance of a constant flow is important, since the endogenous levels of catechol amine in perfused guinea-pig hearts are decreased by increased flow rates (Levitt, Spector, Sjoerdsma & Udenfriend, 1965). Increased perfusion rates have also been shown to increase the loss of [^{3}H]-octopamine from spleen that occurs spontaneously or after splenic nerve stimulation (Fischer, Horst & Kopin, 1965). Finally, the retention of [^{3}H]-noradrenaline by cat spleen is inversely related to the rate of perfusion (Paton & Gillis, 1965). Reference to Table 1 shows that this relationship also applies to the cat heart.

The retention of [^{3}H]-noradrenaline by each heart during two successive infusions under identical conditions did not differ significantly. This finding was similar to that previously reported for cat spleen (Paton & Gillis, 1965) and is, of course, essential if the retention of amine under control and test conditions is to be determined in each

heart. The use of such " internal controls " made possible the recognition (Table 1) of a significant reduction in [^{3}H]-noradrenaline retention during hypothermia despite the fact that the mean values for total retention in the control and test experiments are not quantitatively very different. The finding that there was considerable retention of amine at 29° C was not unexpected, since it has been shown that, while less [^{3}H]-noradrenaline is taken up at temperatures below 37° C by heart slices (Dengler, Michaelson, Spiegel & Titus, 1962), by isolated sympathetically innervated atria (Schneider & Gillis, unpublished) and by " granule " fractions from rat (Potter & Axelrod, 1963) and rabbit heart (Gillis, 1964a), the process of retention is by no means entirely dependent on temperature. During hypothermia, all hearts exhibited pronounced bradycardia. It was, therefore, difficult to compare directly the responses to noradrenaline infusion during hypothermia with those during infusions at 37° C (when the preinfusion rate was much higher). Although the percentage rate increase in response to noradrenaline infusion was considerably increased during hypothermia, true potentiation in these experiments could be based only on a comparison of dose/response curves at each temperature. It can be stated fairly, however, that the elevated response to noradrenaline or to sympathetic stimulation during hypothermia, demonstrated by others (Della Bella *et al.*, 1965 ; Schneider & Gillis, unpublished), could be associated with a decreased tissue uptake of the amine, reflected in the significant reduction of [^{3}H]-noradrenaline retention observed in the present study.

It was somewhat surprising to find that anoxia failed to influence the retention of [^{3}H]-noradrenaline, whether the amine was presented to the heart rapidly (in 1 min) or more slowly (over 20 min). However, using a semiquantitative method, Malmfors (1965) was able to demonstrate uptake of noradrenaline by rat iris *post mortem* (and therefore in the presence of anoxia). The ability of peripheral tissues to retain noradrenaline in the face of pronounced anoxia is not incompatible with the belief (Dengler *et al.*, 1962 ; Iversen, 1963 ; Gillis, 1964b) that the amine is retained mainly as a result of an active (energy dependent) transport mechanism. Such energy requirement may represent only a small drain on the energy reserves of the cell (Dengler *et al.*, 1962). In addition, it must be considered that the source of the energy required for this transport is by no means certain (Csáky, 1965).

Both hypothermia and anoxia produced bradycardia in the perfused heart. However, it is most unlikely that a decreased heart rate *per se* is associated with altered [^{3}H]-noradrenaline retention, since anoxia, which caused a greater degree of bradycardia than did hypothermia, had no obvious effect on the retention of amine.

[^{3}H]-Noradrenaline retention was apparently unaffected at a time when the physiological response to a prolonged infusion of the amine was decreasing (Fig. 1). There can be little doubt that only a very small portion of the infused amine was responsible for increasing the heart rate. Therefore, our failure to show a significant drop in [^{3}H]-noradrenaline retention does not rule out the possibility of an altered local concentration of amine at the catechol amine receptor sites. It has been shown (Kaplan, Gitlow & Smith, 1963) that the retention of [^{3}H]-noradrenaline by the hearts (but not other organs) of rabbits rendered tolerant to narcotic and lethal doses of noradrenaline was significantly decreased. If these animals showed the same depressed cardiovascular response to noradrenaline as did those receiving infusions of noradrenaline (Rosenthale

& Dipalma, 1963), then the decreased [^{3}H]-noradrenaline retention might be due to a difference in the fraction of cardiac output delivered to their hearts (Wurtman, Kopin, Horst & Fischer, 1964) rather than to an altered amine uptake.

It must be considered finally that the observed lack of effect of anoxia or tolerance to the chronotropic action of prolonged infusion on the retention of [^{3}H]-noradrenaline may indicate that the altered reactivity to catechol amines found under these circumstances is due to changes at the receptor and/or the effector cell level. These negative findings could in fact support the contention of Zaimis (1964) that it is incorrect to consider the effect of drugs (and other factors) on adrenergically innervated structures as if they took place only at nerve endings.

SUMMARY

1. The cat isolated heart, perfused with Krebs medium at a constant flow rate, was used to study the retention of [^{3}H]-noradrenaline by a number of conditions.

2. Lowering the temperature of the heart to 29° C significantly ($P<0.001$) decreased [^{3}H]-noradrenaline retention.

3. Increasing the rate of perfusion at 37° C decreased significantly the retention of [^{3}H]-noradrenaline.

4. Anoxia or tolerance to the positive chronotropic effect of noradrenaline infusion failed to influence the retention of the tritiated amine.

5. It is concluded that the potentiated response to noradrenaline that accompanies hypothermia may be linked to a decreased tissue retention of the amine; and that the depressed cardiovascular reactivity to noradrenaline produced by anoxia or by prolonged infusions of the amine is unlikely to be due to any alteration of tissue noradrenaline-binding capacity.

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