Brit. J. Pharmacol. (1962), **18**, 183–189.

EFFECT OF ADRENALINE INFUSIONS ON THE CATECHOL AMINE CONTENT OF CAT AND RAT TISSUES

BY

JUDITH A. HARVEY* AND JOCELYN N. PENNEFATHER†

From the Department of Pharmacology, University of Sydney, N.S.W., Australia

(*Received October* 25, 1961)

Infusions of adrenaline failed to produce a significant increase in the adrenaline and noradrenaline content of cat uterus and kidney and of rat uterus and spleen, using normal and reserpine-treated animals. The adrenaline content of extracts of the organs was assayed on the isolated rat uterus, which was stimulated electrically; the noradrenaline content was assayed on the blood pressure of the pithed rat. The tissues studied contained relatively small quantities of adrenaline and this was not decreased by reserpine. Our results show a difference between the stores of adrenaline and noradrenaline in these tissues; the noradrenaline stores are larger, they are depleted by reserpine, and they are increased by an infusion of noradrenaline.

Although adrenaline is capable of enhancing responses of sympathetically innervated tissues to tyramine and to sympathetic nerve stimulation, for instance, on the blood perfused vessels of the dog's hind limb (Burn, 1932a & b), it is less effective than noradrenaline in this respect. Thus Burn & Rand (1960a) found that noradrenaline affords a greater and a longer-lasting enhancement of the responses of the dog's hind limb to nerve stimulation than does adrenaline. Similarly, these authors (Burn & Rand, 1960b) found that noradrenaline is the more effective substance in restoring blood pressure responses to tyramine in reserpine-treated cats and rats.

In 1932 Burn put forward the suggestion that sympathetically innervated tissues take up adrenaline from the circulation, thus replenishing a store on which sympathetic nerve impulses impinge and release this amine. Later, Burn & Rand (1960a) made a similar suggestion that such uptake occurs also with noradrenaline. Direct evidence supporting the latter suggestion has been provided by the experiments of Pennefather & Rand (1960), who found that an infusion of noradrenaline increases the noradrenaline content of cat kidney and uterus. They also showed that dopamine and L-dopa increased the noradrenaline content, but they did not investigate the ability of an adrenaline infusion to increase the catechol amine content of these tissues. Since adrenaline is less effective than noradrenaline in restoring responses to sympathetic nerve stimulation, it may also be less effective in replenishing the tissue store.

* Present address: Department of Physiology and Pharmacology, Chelsea College of Science and Technology, London, S.W.3.

† Present address: Department of Pharmacology, School of Pharmacy, Brunswick Square, London W.C.1.

The ability of adrenaline to enter sympathetically innervated tissues has been demonstrated by Axelrod, Weil-Malherbe & Tomchick (1959), using [^{3}H]-adrenaline. However, this finding does not necessarily imply that an infusion of adrenaline *increases* the amounts of adrenaline in tissues. The present experiments with adrenaline have been carried out using a method similar to that of Pennefather & Rand (1960), to determine directly whether an infusion of adrenaline increases the adrenaline content of tissues. In view of the possibility that demethylation of adrenaline might occur in these experiments, we have also measured noradrenaline tissue levels after the adrenaline infusions.

METHODS

Infusions of adrenaline in cats. The method used to investigate uptake of adrenaline in cat tissues was essentially similar to that described by Pennefather & Rand (1960) for other catecholamines. Cats were made spinal and eviscerated. Blood pressure was recorded from one carotid artery and a 1 mm polythene tube inserted into the other to allow removal of blood. After ligating the renal vessels at the hilus the right kidney was dissected free and excised. In female cats, one uterine horn was removed after ligating the ovarian artery and vein. A blood sample, 4 to 5 ml., was withdrawn into a polythene centrifuge tube containing 100 i.u. heparin. Adrenaline, 1 mg, dissolved in 14.3 ml. of 0.9% sodium chloride solution at *p*H 4.5, was infused for 40 min into a small vein in the foreleg. Twenty min after disconnecting the infusion apparatus the left kidney and the other uterine horn were removed and a second blood sample taken.

The blood samples were centrifuged for 5 min at 5,000 rev/min immediately after collection and the decanted plasma stored at $-10°$ C until assayed. The kidneys and uterine horns were cleared of surrounding tissue, weighed and stored in the deep freeze, not more than 10 min elapsing after their removal.

Infusions of adrenaline in rats. Female rats were pithed under ether, and blood pressure was recorded by inserting into a carotid artery a polythene tube connected to a strain-gauge pressure transducer (Statham Laboratories, type P 23 Db). The output from the transducer bridge circuit was fed into a DC amplifier connected to an ink writing recorder (compare Pennefather & Rand, 1960). A jugular vein was cannulated. The abdomen was opened and one uterine horn was removed as described for cats. Adrenaline, 80 μg, in a volume of 2 ml. (0.9% sodium chloride solution adjusted to *p*H 4.5), was infused during 20 min. Twenty min after disconnecting the infusion apparatus the second uterine horn was removed. In some experiments the spleen was removed either before or after the infusion of adrenaline.

Assay of catecholamines. Tissues were extracted as described by Burn & Rand (1959). The extracts were then assayed for their noradrenaline content on the blood pressure of the pithed rat, and for their adrenaline content on the electrically stimulated rat uterus preparation (Harvey & Pennefather, 1962). A uterine horn was removed from a rat which had received 100 μg stilboestrol intramuscularly 30 hr previously. This was suspended in a 10 ml. organ bath containing either the bathing solution described by Gaddum, Peart & Vogt (1949) or Krebs solution containing 1/10 of the usual calcium concentration. Oxygen was bubbled through the bathing solution which was maintained at 29° C. Contractions were recorded by means of an isotonic lever. Regular contractions of the uterus were obtained by stimulating for 10 to 20 sec every 2 min with 4 to 8 V AC pulses (50 c/s) applied longitudinally by means of silver electrodes. At intervals, standard doses of adrenaline or aliquots of extracts were added to the bath 45 sec before stimulation. Atropine (10^{-7}) was added to the bath in all assays, and when rat spleen extracts were assayed 2-bromolysergic acid diethylamide (10^{-8}) was also added. The electrically induced contractions of the uterus were inhibited by as little as 1 to 5 pg adrenaline, that is, a concentration in the bath of 1 to 5×10^{-13}. The preparation was at least 10,000 times less sensitive to noradrenaline.

Reserpine treatment. Cats were treated with reserpine by intraperitoneal injection of 3 to 4 mg reserpine in 20% ascorbic acid on each of two days and were used on the third day. Rats received a total intraperitoneal dose of 6 mg/kg during the two days prior to use.

RESULTS

Effect of adrenaline infusions on catechol amine content of cat tissues

Uterus. The estimation of the adrenaline content of uterine horns on the rat uterus preparation is illustrated in Fig. 1. In this assay 0.4 ml. of an extract of

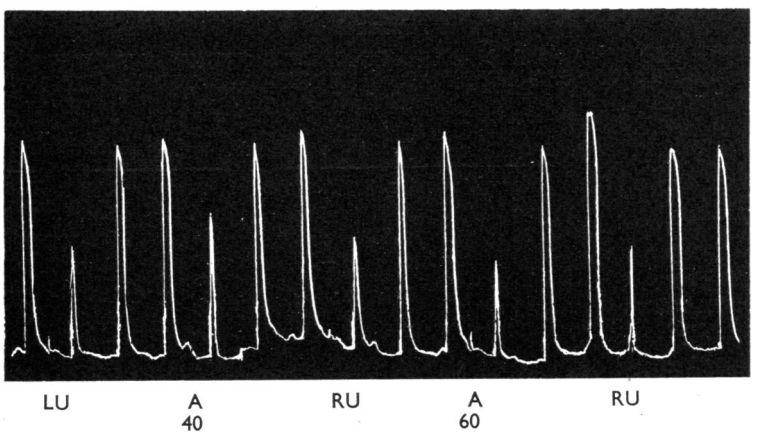

Fig. 1. Rat uterus stimulated electrically with 4 V 50 c/s; duration 12 sec, interval 2 min. LU= inhibition produced by 0.4 ml. of an extract of the left uterine horn removed from reserpine-treated cat 20 min after infusing 1 mg adrenaline. RU=0·4 ml. of a uterine extract made before the infusion. Both extracts produced inhibitions of a size intermediate between that produced by 40 and 60 pg adrenaline at A.

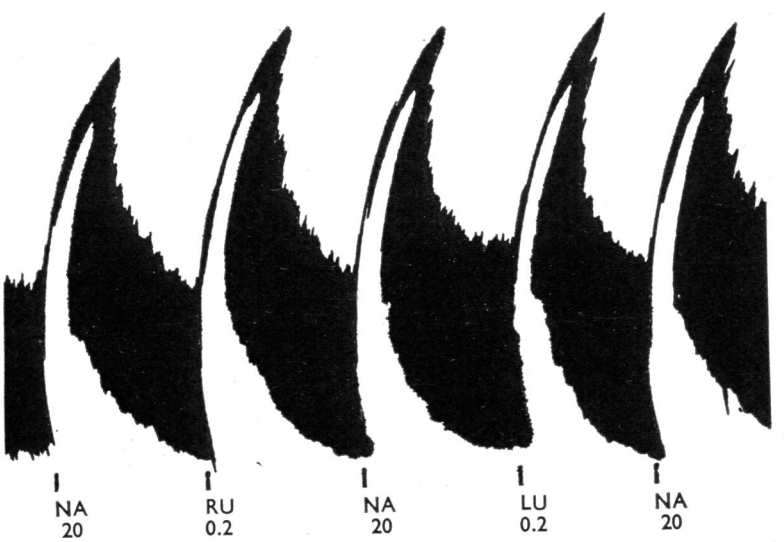

Fig. 2. Rat blood pressure recorded with a transducer manometer. RU=response to 0.2 ml. of an extract of the right uterine horn (removed from the cat before the infusion) equal to 20 ng noradrenaline at NA, and to LU=0.2 ml. of an extract similarly prepared from the left uterine horn, removed from the cat 20 min after the infusion.

one horn of the uterus taken from a reserpine-treated cat after the adrenaline infusion was equipotent with the same volume of an extract made from the other horn taken before the infusion. Fig. 2 illustrates the estimation on the rat blood pressure of the noradrenaline content of uterine horns taken before and after infusing adrenaline. Again, the extracts were equipotent.

Table 1 gives the adrenaline and noradrenaline equivalents of cat uterus before and after infusing 1 mg adrenaline. No significant increase occurred in either the

TABLE 1

NORADRENALINE AND ADRENALINE CONTENTS (NG/G) OF UTERUS BEFORE AND AFTER INFUSING 1 MG ADRENALINE IN NORMAL AND RESERPINE-TREATED CATS

	Noradrenaline			Adrenaline		
	Before	After	Increase	Before	After	Increase
Normal cats	500	500	0	1·5	1·5	0
	75	75	0	0·4	0·3	0·1
	375	350	−25	1·5	1·5	0
	425	425	0	0·8	1·0	0·2
Reserpine-treated cats	42	50	8	0·6	0·6	0
	13	13	0	2·0	2·0	0

noradrenaline or adrenaline contents of the uterus after infusing adrenaline. Adrenaline comprised only a very small proportion of the total catechol amines present in the uterus in untreated cats. Though the levels of noradrenaline in the uterine horns were reduced by reserpine treatment, the amounts of adrenaline in the reserpine-treated tissues were not significantly different from those present in the untreated cats.

Kidney. The adrenaline and noradrenaline contents measured before and after infusing adrenaline are shown in Table 2. As in the uterus, adrenaline was present

TABLE 2

NORADRENALINE AND ADRENALINE CONTENTS (NG/G) OF KIDNEY BEFORE AND AFTER INFUSING 1 MG ADRENALINE IN NORMAL AND RESERPINE-TREATED CATS

	Noradrenaline			Adrenaline		
	Before	After	Increase	Before	After	Increase
Normal cats	200	275	75	0·3	15·0	14·7
	125	125	0	2·0	1·6	−0·4
	—	—	—	0·1	0·5	0·4
	225	225	0	2·5	2·5	0
	—	—	—	1·0	1·0	0
Reserpine-treated cats	—	—	—	1·5	1·5	0
	75	75	0	2·5	2·5	0

in the kidney in very small amounts and the infusion of adrenaline did not raise its content, except in one cat. In this cat an increase in noradrenaline content was also recorded, but this was not reproduced in the other tissues in which noradrenaline levels were measured. As in the uterus, the noradrenaline content of the kidney was reduced by reserpine treatment, but the adrenaline content was not affected.

Fig. 3 shows the pressor responses to tyramine in one of the reserpine-treated cats before and after infusing 1 mg adrenaline. Although the infusion itself produced a clear pressor response, it did not enhance the response to tyramine. This failure may be in part related to the failure of the infusion to increase the catechol amine content of the tissues studied.

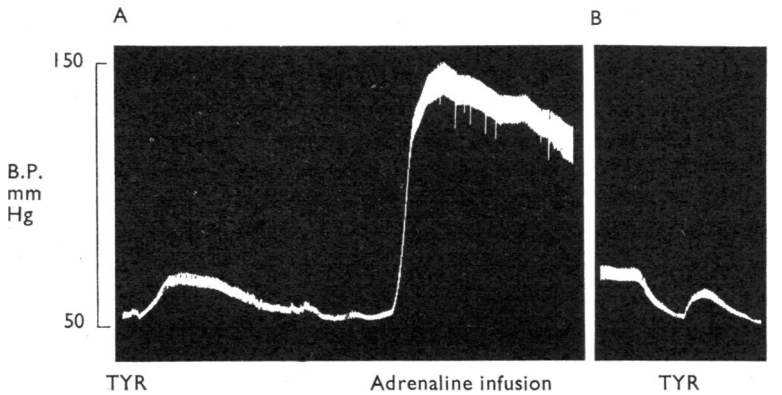

Fig. 3. Record of blood pressure of a reserpine-treated cat. A, Tyramine 5 mg (TYR) produced only a small pressor response. The infusion of 1 mg adrenaline caused a large pressor effect. B, Response to 5 mg tyramine 10 min after the end of the infusion produced a response no greater than that produced before infusing adrenaline.

Plasma. Noradrenaline levels were not raised after infusing adrenaline in a normal cat nor in the two reserpine-treated cats. Plasma adrenaline levels were not estimated.

Effect of adrenaline infusions on adrenaline content of rat uterus and spleen

The adrenaline contents of uterine horns taken before and after infusing adrenaline are shown in Table 3. In two rats the amount present before the infusion was below the lower limit of sensitivity of the assay. However, in all four normal rats

TABLE 3

ADRENALINE CONTENT (NG/G) OF UTERUS BEFORE AND AFTER INFUSING 80 μG ADRENALINE IN NORMAL AND RESERPINE-TREATED RATS

	Before	After	Increase
Normal rats	0·30	0·98	0·68
	0·30	2·04	1·74
	<0·13	0·13	+
	<0·01	0·01	+
Reserpine-treated rats	2·31	2·21	−0·1
	0·02	0·02	0

the infusion increased the adrenaline content of the uterus. Indeed, in one rat there was a threefold increase and in another a sixfold increase. Nevertheless, the total amounts of adrenaline present in the tissues after the infusion were still minute when considered in relation to the comparatively large dose infused. Thus less than 0.0000005% of this was accounted for by uptake in the uterus (which weighs

150 to 300 mg). In the two reserpine-treated rats no increase in the adrenaline content of the uterus occurred after the infusion.

In some experiments the adrenaline contents of rat spleens taken before and after the infusions were estimated. Two spleens taken before the infusion had adrenaline contents of 1.0 and 0.25 ng/g. In four spleens taken after infusions the levels were 1.0, 1.5, 2.5 and 1.0 ng/g.

DISCUSSION

The tissues we have studied contained relatively little adrenaline, and this was not reduced by reserpine treatment. The infusion of large amounts of adrenaline to normal and reserpine-treated animals produced no significant increase in either the adrenaline or noradrenaline contents. This failure of adrenaline to increase the catechol amine contents of the tissues provides a sharp contrast to the situation obtaining when noradrenaline is infused. Pennefather & Rand (1960) after infusing 1 mg noradrenaline have recorded increases in the noradrenaline levels in kidney and uterus of untreated and reserpine-treated cats. Infusions of noradrenaline also raise the noradrenaline contents of rat spleen and uterus (Muscholl, 1961 ; Farrant, Harvey & Pennefather, 1962).

Initially, our results seem to conflict with those obtained by Axelrod *et al.* (1959) in their experiments with [^{3}H]-adrenaline. They found that radioactivity ascribable to adrenaline was detected in many tissues immediately after the infusion. Our estimations were not made until 20 min after ceasing the infusion. The possibility cannot be excluded that the measurements made by Axelrod *et al.* (1959) were of the degree of exchange of labelled adrenaline with that already present in the tissues, rather than of an increase in the total tissue adrenaline level. Another explanation for the apparent discrepancy between our results and those of Axelrod *et al.* (1959) is that the various tissues studied may differ in their relative capacities to retain infused adrenaline. For example, the heart stores adrenaline for periods of more than an hour (Raab & Gigee, 1955 ; Axelrod *et al.*, 1959 ; Muscholl, 1961).

Our finding that infused adrenaline is not retained by tissues which do retain infused noradrenaline underlines the ability of a single tissue to discriminate precisely between the two amines. The possibility that the two amines are stored differently in tissues has been raised by von Euler & Purkhold (1951) and by Hillarp & Hökfelt (1953, 1955). The former workers found that section of postganglionic sympathetic fibres reduced the noradrenaline levels in sheep spleen, kidney and salivary glands, but did not alter those of adrenaline. The differential depletion of noradrenaline by reserpine in cat kidney and uterus is in line with their results.

The fact that adrenaline is less effective than noradrenaline in increasing tissue catechol amine levels may explain the findings of Burn & Rand (1960a & b) that it is also less effective in enhancing the responses of tissues to sympathetic nerve stimulation and to injected tyramine. These results with adrenaline lend further emphasis to their postulate that it is the size of the noradrenaline store which modulates the responses of sympathetically innervated tissues.

This work was undertaken during the tenure of the Drug Houses of Australia Research Fellowship in Experimental Pharmacology by J. N. P., and the Burroughs Wellcome & Co.

(Aust.) Research Fellowship in Experimental Pharmacology by J. A. H. We are grateful to Dr M. J. Rand for suggesting this work to us, and to Professor R. H. Thorp for his continued interest and the provision of facilities. We would also like to thank Dr G. Etter, of Sandoz Australia Pty., for a gift of 2-bromolysergic acid diethylamide.

REFERENCES

AXELROD, J., WEIL-MALHERBE, H. & TOMCHICK, R. (1959). The physiological disposition of H^3-epinephrine and its metabolite metanephrine. *J. Pharmacol. exp. Ther.*, **127**, 251–256.

BURN, J. H. (1932a). On vasodilator fibres in the sympathetic, and on the effect of circulating adrenaline in augmenting the vascular response to sympathetic stimulation. *J. Physiol. (Lond.)*, **75**, 144–160.

BURN, J. H. (1932b). The action of tyramine and ephedrine. *J. Pharmacol. exp. Ther.*, **46**, 75–95.

BURN, J. H. & RAND, M. J. (1959). The cause of the supersensitivity of smooth muscle to noradrenaline after sympathetic degeneration. *J. Physiol. (Lond.)*, **147**, 135–143.

BURN, J. H. & RAND, M. J. (1960a). The relation of circulating noradrenaline to the effect of sympathetic stimulation. *J. Physiol. (Lond.)*, **150**, 295–305.

BURN, J. H. & RAND, M. J. (1960b). The effect of precursors of noradrenaline on the response to tyramine and sympathetic stimulation. *Brit. J. Pharmacol.*, **15**, 47–55.

FARRANT, J., HARVEY, J. A. & PENNEFATHER, J. N. (1962). The influence of phenoxybenzamine on the uptake of noradrenaline by cat and rat tissues. *J. Physiol. (Lond.)*, **159**, 49–50*P*.

GADDUM, J. H., PEART, W. S. & VOGT, M. (1949). The estimation of adrenaline and allied substances in the blood. *J. Physiol. (Lond.)*, **108**, 476–481.

HARVEY, J. A. & PENNEFATHER, J. N. (1962). Uptake of adrenaline by cat tissues. *J. Physiol. (Lond.)* (In the press).

HILLARP, N. A. & HÖKFELT, B. (1953). Evidence of adrenaline and noradrenaline in separate adrenal medullary cells. *Acta physiol. scand.*, **30**, 55–68.

HILLARP, N. A. & HÖKFELT, B. (1955). Histochemical demonstration of noradrenaline and adrenaline in the adrenal medulla. *J. Histochem. Cytochem.*, **3**, 1–5.

MUSCHOLL, E. (1961). Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. *Brit. J. Pharmacol.*, **16**, 352–359.

PENNEFATHER, J. N. & RAND, M. J. (1960). Increase in noradrenaline content of tissues after infusion of noradrenaline, dopamine and L-DOPA. *J. Physiol. (Lond.)*, **154**, 277–287.

RAAB, W. & GIGEE, W. (1955). Specific avidity of the heart muscle to absorb and store epinephrine and norepinephrine. *Circulation Res.*, **3**, 553–558.

VON EULER, U. S. & PURKHOLD, H. (1951). Effect of sympathetic denervation and noradrenaline and adrenaline content of spleen, kidney and salivary glands in the sheep. *Acta physiol. scand.*, **24**, 212–217.